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DETERMINATION OF THE LEVEL OF ANDROGENESIS IN TOBACCO

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ABSTRACT

Androgenesis is the newest and most secure method to obtain haploid plants *in vitro*, where vegetative or generative nucleus of a pollen grain is stimulated to develop into a haploid individual. There are different possibilities for regeneration and formation of microspores in various genotypes of tobacco. In this case, the level of androgenesis was investigated in three tobacco genotypes. NN-media was used as a basic medium for microspores development and MS-media for rhizogenesis and organogenesis, together with adequate combinations of plant hormones (JAA, BAP, adenine, glutamine and kinetine).

Key words: haploids, tobacco, androgenesis, medium

ОДРЕДУВАЊЕ НА СТЕПЕНОТ НА АНДРОГЕНЕЗА КАЈ ТУТУНОТ

Методата на андрогенеза претставува најнова и најсигурна метода за добивање на хаплоидни растенија in vitro, каде вегетативното или генеративното јадро од поленовото зрно се стимулира за добивање на хаплоидни единки. Можноста за регенерација и формирање намикроспори кај одредени генотипови од тутунските растенија се различни, за таа цел во овој труд вршевме испитувње на степенот на андрогенеза кај три различни генотипови тутун. Како основен медиум за развиток на микроспорите беше користен NN- медиумот (Nitch J. P. и Nitch C., 1969), додека за ризогенеза и органогенеза MS-медиум(Murashige T., и Skoog F., 1962), како и соодветни комбинациина растителни хормони и тоа: ЈАА, БАП, аденин, глутамин и кинетин.

Клучни зборови: андрогенеза, медиум, генотипови, тутун

INTRODUCTION

Haploid plants can be obtained by isolation of anthers *in vitro* in two ways:

- direct , with formation of embryoids from the pollen grain (microspore), and

- indirect, with callus development and formation of haploid embryoids or adventive buds [6].

The latter type of development is unsuitable, because callus as a starting material is of heterogenic nature (haploids and diploids).

Tobacco is an ideal plant for obtaining haploid cultures in direct way. Tobacco cultures produce an explosion of haploids, which are now used in hybridization processes. Some authors stimulated the production of female gametes (*gynogenesis*) or male gametes (*androgenesis*) in direct haploid individuals. They came to conclusion that in gynogenesis, which is carried out *in vivo*, female cells are stimulated to grow without fertilization. In androgenesis, which is carried out only *in vitro*, vegetative or generative nuclei from pollen grains are stimulated to develop haploid plants without fertilization. The literature on androgenesis *in vitro* [6, 1] clearly shows that the Solanaceae family species are capable of regeneration of haploids from isolated anthers.

The goal of this paper was to investigate the genetic potential of some newly created lines in vitro, using the method of androgenesis.

MATERIALS AND METHODS

Anthers from three oriental tobacco lines were used for determination of the level of androgenesis: Line 137, Line 147 and Line 208. We used Nitsch-Nitsch (NN),[5] as a basal medium, and Murashige-Skoog (MS), [4] medium was used for rhizogenesis and organogenesis, together wit adequate combinations of plant hormones like JAA, BAR, adenine, glutamine and kinetin. Sterilization of buds was made with 2% HgCl and 70% alcohol, and they were finally washed in sterilized water. Androgenetic potential was evaluated by the classification of Mityko and Fari [3]:

- poor androgenetic potential - up to 5% embryogenetic anthers
- average androgenetic potential - 5 - 10% embryogenetic anthers
- good androgenetic potential - 15-30% embryogenetic anthers
- high androgenetic potential - over 30% embryogenetic anthers

RESULTS AND DISCUSION

Androgenesis can be induced in many agricultural plants, but the ability of some species for successful microspores propagation is often limited and depends on the genotype itself, i.e. on the variety. The choice of treatment that should be applied at *in vitro* conditions is based on the immense literature data on anthers and their regeneration [2], paying equal attention to the specificity of each genotype for regeneration in practice.

According to the results of induction of haploid embryos from anthers of the investigated tobacco lines (Table 1), line L.132 has the best embryogenetic potential and the highest percentage of anthers (32%) from those set up for regeneration.

Table 1 . Induction of haploid embryos from anthers in tobacco

Lines	Number of anthers	Embryogenetic anthers %	Callus anthers %	Embryogenetic potential
Line 137 F2	40 ± 4	32 ± 2,1	5 ± 1	High
Line 147 F2	36 ± 2	24 ± 3,0	2 ± 2	Good
Line 208 F3	40 ± 1	24 ± 2,5	3 ± 1,5	Good

Table 2. Morphological properties of haploid embryos from anthers in tobacco

Lines	Number of haploid plants	Plant height, cm	Root length
Line 137 F2	55 ± 0,5	3,4 ± 0,5	1,2 ± 0,4
Line 147 F2	24 ± 1,2	5,0 ± 0,7	1,0 ± 0,9
Line 208 F3	34 ± 2,0	4,8 ± 1,2	1,5 ± 1,5

Fig.1 Introduction of haploid emryos anther in tobacco

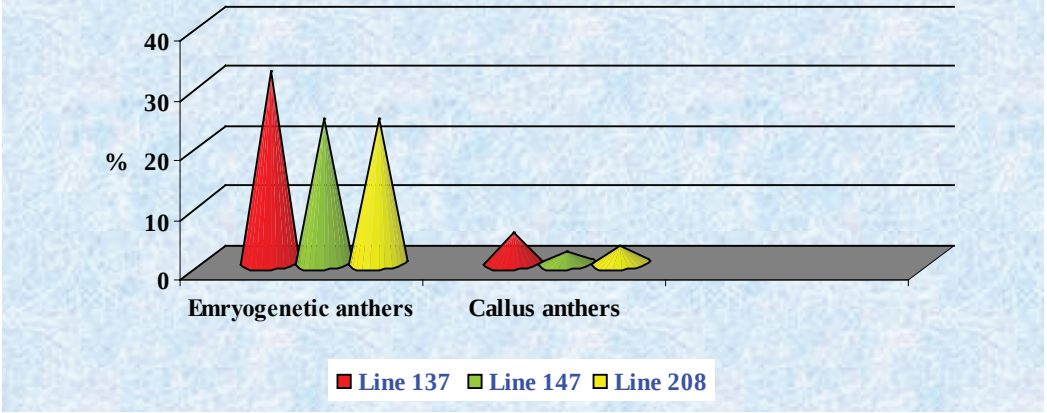
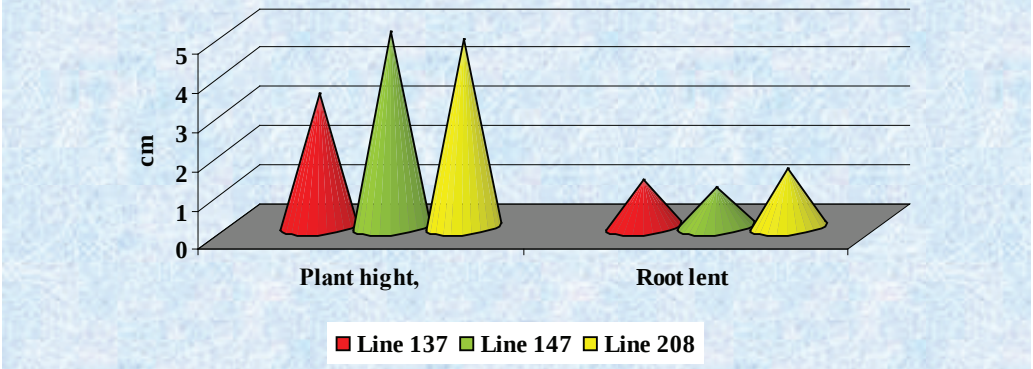


Fig. 2. Morphological properties of haploid embryos from anthers in tobacco



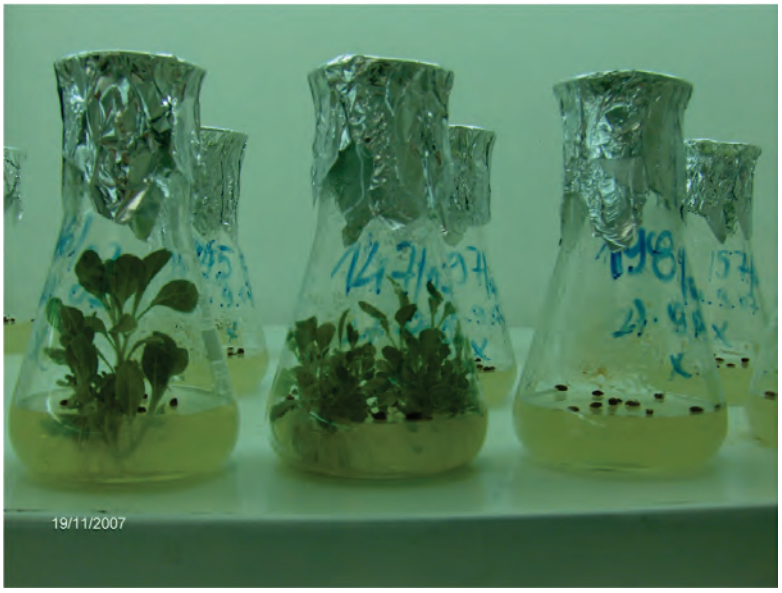


Photo 1. Haploid tobacco plants

Referring to morphological characters of the haploid embryos (Table 2), it can be stated that L. 137 has the highest number of haploid plants, which confirms that it possesses the best embryogenetic potential among all other tobacco lines investigated, according to the classification of Mityko & Fari [3].

CONCLUSION

According to the results, genotypes included in investigations have different abilities for embryoids formation and the callus formation in all of them is minimum.

- The greatest genetic potential was noted in the line L 137 (32%), which yielded the highest number of haploid plants (55).

- According to the classification of Mityko and Fari [3], the androgenetic potential is good in lines L 147 and L 208 and high in L 137.

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TRANSMISION OF RESISTANCE TO TOBACCO MOSAIC VIRUS (TMV) IN SOME ORIENTAL TOBACCO VARIETIES

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ABSTRACT

The aim of investigation was to study the transmission of TMV resistance in some commercial varieties of oriental tobacco by the method of backcross hybridization. Investigation included resistant introduced oriental varieties (AA) and one susceptible variety (aa). In relation to other qualitative and productional characteristics, the latter variety was good for buyers. In F_1, resistant heterozygous progeny (Aa) was obtained, which was back-pollinated with pollen of the recipient variety (aa). In BC_1, as well as in other generations up to BC_4 and BC_5, resistant plants, phenotypically more similar to the recipient variety, were pollinated again with the pollen of this variety. Infective juice from diseased plants was used for inoculation of plants from hybrid generations, using the method of Ternovskiy.

Key words: tobacco, oriental varieties, transmission of TMV resistance, backcrossing

ПРЕНЕСУВАЊЕ НА ОТПОРНОСТА КОН ОБИЧНИОТ МОЗАИК ВИРУС (TMV) КАЈ НЕКОИ ОРИЕНТАЛСКИ СОРТИ ТУТУН

Целта на овие истражувања е да се проучи и прикаже начинот на пренесување на својството отпорност кон обичниот мозаик вирус (TMV) кај некои комерцијализирани ориентални сорти тутун по методот на повратно вкрстување. Како почетен материјал при вкрстувањето се користени странски ориентални сорти отпорни на вирусот (АА) и неотпорни сорти-примачи на својството отпорност (аа), кои во однос на другите квалитетни и производни својства се добри за откупувачите. Во F_1 е добиено отпорно хетерозиготно потомство (Аа), кое повратно се наситува со полен од сортата примач (аа). Во првата повратна генерација (BC_1), како и во останатите генерации до BC_4 и, BC_5, земани се отпорни растенија кои по својот фенотип се поблиску до сортата-примач и повторно беа наситувани со полен од сортата-примач на ова својство. За инокулација на растенијата од одделните хибридни генерации се користеше инфективен сок од мозаични тутунски растенија, а заразувањето е извршено по методот на Терновский.

Клучни зборови: тутун, ориентални сорти, пренесување на отпорност на TMV, повратно вкрстување

INTRODUCTION

Tobacco diseases, regardless of the pathogen origin (viruses, bacteria, fungi) cause severe loses in tobacco yield and quality each year. Tobacco mosaic is the most widely spread viral disease in the world. When infestation occurs immediately after transplanting, in some plots it can attack 50 - 100% of plants, reducing the yield for 30% and total value of tobacco for 50% (13, 9). The adaptability, thermoresistance, unusual ability for change and rapid multiplying in leaf tissue make this virus hard to control (14). In addition, the disease appearance can not be prevented by chemical products. The problem can be solved only by creation of new resistant varieties and their implementation in mass production (5, 8, 12,14).

Having in mind that commercial oriental varieties of the type Prilep are susceptible to tobacco mosaic virus (TMV), the aim of these investigations was to study the transmission of resistance to the virus in variety Prilep 65/94, using the backcross method.

MATERIAL AND METHODS

Trials were set up in field and Biolaboratory of Tobacco Institute-Prilep, in the scope of the project "Creation of TMV resistant oriental tobacco varieties", financed by the Ministry of Education and Science of Macedonia, Dimitrieski et al.(4).

Oriental variety Nevrokop 1146 (photo 1), created by intervariety hybridization of Nevrokop 261 and Nevrokop B12 in Tobacco Trial Station Goce Delcev – Bulgaria (5), was used as starting material. All these varieties were resistant to TMV. Crossing was made with oriental variety Prilep 65/94 (non-resistant to TMV – photo 2), created in Tobacco Institute-Prilep. Parental varieties were selected on the basis of earlier investigations and the backcross method was used in the selection process. Hybrid progenies of F_1, as well as progenies of backcross generations BC_1, BC_2, BC_3 and BC_4 were grown on sufficient area and with sufficient number of individuals, as required by the selection program. Resistant plants, phenotypically closer to the receiver variety, were selected from F_1 progenies and backcross generations BC_1 – BC_4 and backcrossed with the same variety. In future generations, the trait of TMV resistance was stabilized according to the mode of monohybrid dominant inheritance. Juice from mosaic-infected tobacco plants was used for inoculation, according to the method of Ternovsky 1965, (cit. by Tranceva, 15). The juice from infected leaves was heated for 12 minutes in water bath at 80 0 C, in order to inactivate all other viruses, e.g. cucumber mosaic virus (CMV) and Potato Virus Y (PVY). Observations were made between the 7th and 10th day of inoculation, after the appearance of local necrotic spots on resistant plants and mosaic patterns on the leaves of susceptible plants.

RESULTS AND DISCUSSION

In the process of selection, TMV resistant variety Nevrokop 1146 (AA) was used as maternal component and non-resistant Prilep 65/94 (aa) as paternal component. In hybrid progeny of F_1 resistant heterozygous plants (Aa) were obtained and all hybrids showed necrotic reaction after infestation, indicating that the resistant allele is dominant and the non-resistant one is recessive. The dominant inheritance of this trait was also confirmed by other authors. Holmes, Kostov and Ternovskiy obtained homozygous forms of tobacco (N. tabacum) which succeeded to localize TMV. Holmes was the first to report from genetic aspect that one gene is responsible for localization of the virus, which usually appears as dominant (cited by Kostov, 10).

According to S. Stoyanov, Gelemerov (5), Kostov crossed the resistant form N. tabacum var. viri (N. tabacum x N. glutinosa) with Basma 36 and noted dominant inheritance of this trait (existence of local necrotic reaction). Ternovskiy used TMV resistant gene from N. glutinosa to create the varieies

Dubek 7, Dubek 566, Trapezond 161 etc. He reported that Shabanov, Lulov and Manolov also worked on TMV resistant varieties. Petkova (14) investigated three TMV resistant tobacco varieties which transmitted this trait in F_1 with dominant inheritance. She reported that the same statement was confirmed by Manun (1981). Three well developed plants from F_1 progeny (Aa) with uniform phenotypes were backcrossed with pollen from the receiver variety (aa)..

In BC_1 progeny, 50% TMV resistant heterozygous plants and 50% non-resistant plants were obtained, i.e. the genotypic ratio was 1Aa : 1aa. This is in accordance with one of the basic Mendel's premises, that individuals with Aa genotype form two types of equally represented gametes (Scheme 1). It was confirmed by many other authors and in various crops (2, 3, 11, 4, 1, etc). Adequate number of plants phenotypically closest to the receiver variety were selected from each resistant variety (Aa) and backcrossed with pollen of the same variety (aa).

In BC_2 progeny, resistant and non-resistant plants were obtained after the infestation, just like in BC_1. Three best developed plants from each resistant variety (Aa), phenotypically closer to the receiver variety, were selected and backcrossed with pollen of the same variety (aa).

The same procedure was also applied in progenies of BC_3 and BC_4 generations, with possibility for its further application in eventual backcross hybridizations in future.

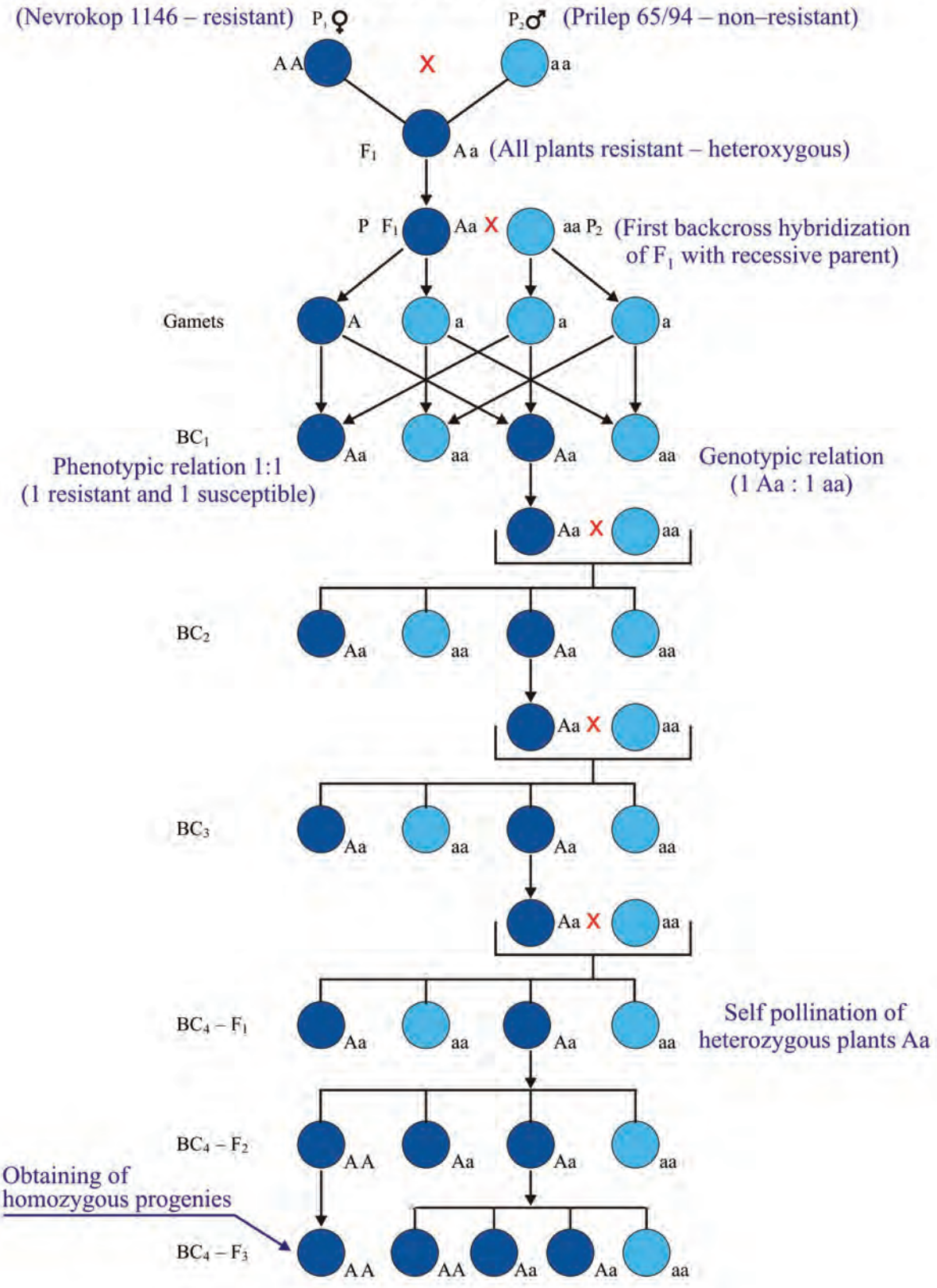
In BC_4 genotype, the receiver variety (aa) is represented five times. With continuous elimination of adverse traits from the donor variety and parallel maintenance of desired traits from the receiver variety, we concluded that this number of backcrossings was sufficient. Therefore, the progeny of this generation was taken as F_1 and two well developed resistant plants, which were the best representatives of the receiver variety, were selected from it. After that, stabilization of the trait resistance to TMV was made according to the scheme of monohybrid dominant inheritance.

According to Gornik (6), the receiver variety can be renewed with less than 6 backcross generations, which depends not only on the plants selected for backcrossing but also on the parents used in investigation. It should be also mentioned that the resistance to diseases to some varieties is more easily transmitted than to others. The same author (quote from Lukas, 1965), reported that only two backcrossings will be enough for transmission of the resistance to blue mold, by further use of the method of individual selection, with obligatory testing of the progenies obtained from the selected resistant plants.

Photo 1. Nevrokop 1146

Photo 2. Prilep 65/94

Shema 1. Transmission of resistance to TMV by the method of backcrossing



CONCLUSIONS

Investigations on transmission of the trait TMV resistance by the use of backcross method in hybridization between some resistant varieties and non-resistant oriental varieties of the type Prilep led us to the following conclusions:

1. Resistance to TMV in F_1 generation is obtained through dominant inheritance.

2. The monohybrid dominant inheritance in oriental tobacco varieties allows complete control and successful transmission of this trait by the backcross method.

3. The selection scheme applied in our investigations proved to be very practical and effective. It can be used for transmission of resistance in all non-resistant commercial varieties without any adverse effect on other qualitative traits as well as for transmission of some other traits inherited by monohybrid dominant mode.

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STUDIES ON THE INHERITANCE OF THE NUMBER OF LEAVES IN THE CROSSES OF VIRGINIA TOBACCO (NICOTIANA TABACUM L.)

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ABSTRACT

The character and extent of the genetic interactions were determined by applying hybridological analysis as well as by the number of genes differentiating between the initial parent forms and expressions of heterosis and transgression referring to the character number of leaves, the objective being the selection of desired genotypes in six hybrid Virginia tobacco populations, including the best introduced varieties from the USA. The results of the survey of P_1, P_2, F_1 and F_2 proved that the character number of leaves in Virginia tobacco crosses is inherited overdominantly, always in the direction of the parent displaying a higher value of the character. The number of genes influencing the manifestation of the studied character which differentiated between the parents varied from 1 to 12. The strongly pronounced positive epistasis reinforces the phenotype expression of the character "number of leaves per plant". Significant positive heterosis was observed in 3 of the crosses.

The high values determined for the inheritance coefficient and for effective mass selection of phenotypes show that the selection of genotypes characterized by higher number of leaves will be more effective in the sooner hybrid generations.

Key words: Virginia tobacco, genetic analysis, inheritance, hereditability, transgression, heterosis.

ПРОУЧУВАЊЕ НА НАСЛЕДУВАЊЕТО НА БРОЈОТ НА ЛИСТОВИ КАЈ КРСТОСКИТЕ ТУТУН ОД ТИПОТ ВИРЏИНИЈА

Со примена на анализата на хибридизација во природата се одредува степенот на генетската взаемност, бројот на гените по кои се разликуваат основните родителски форми, како и појавата на хетерозис и трансгресија во однос на бројот на листовите. Истражувањата се изведени со шест хибридни популации тутун од типот вирџинија во кои учествуваат најдобрите интродуцирани сорти од САД. Резултатите од истражувањето на P1, P2, F1 и F2, покажуваат дека бројот на листови кај тутунот од типот вирџинија секогаш се наследува доминантно во насока на родителот со поголем број на листови. Бројот на гените кои влијаат врз истражуваната појава по која се разликуваат родителските компоненти се движи од 1 до 12. Силно изразената позитивна епистаза ја засилува фенотипската експресија на својството број на листови од растение. Сигнификантен хетерозис е забележан во три од испитуваните крстоски..

Утврдените високи вредности на коефициентите за наследност и за ефикасност на масовниот избор на фенотипови покажуваат дека изборот на генотипови со поголем број на листови ќе биде поефикасен во пораните хибридни генерации

Клучни зборови: тутун вирџинија, генетска анализа, наследување, наследност, трансгресија, хетерозис.

INTRODUCTION

The number of tobacco leaves is important variety characteristic and is of great significance to the yield (Tomov, 1985; 1986; 1991).

The heterosis in Virginia tobacco finds wide application. Heterotic varieties in Bulgaria have been cultivated since 1973. The heterotic varieties still cover big area under this type of tobacco. Virginia 0454 and Virginia 0514 are the basic varieties in Bulgaria.

Some reports on the inheritance of the number and size of leaves in Virginia and Burley tobacco crosses show that these characters are governed by additive genes (Tchintchev, 1979; Chang, E.Y., C.C. Shyu 1976; Legg, P. D., G. B. Collins. 1974; Shyu, C. C., D. C. Lai, E. Y. Chang. 1975). In investigations of dark tobaccos, Torrecila and Barroso (1980) have also pointed out that additive gene effects is of the greatest importance. Sastry and Prasada Rao, (1980) reported that dominant and additive gene effects are of the highest significance. Chang et al. (1980); Sastry and Prasada Rao (1980) noted that dominant gene effects prevails for the number of leaves in Burley tobacco crosses. Noveva et al. (1984) reported that the inheritance in F_1 is overdominant, and the highest influence was obtained by the epistasis gene effects.

Naumovski (1988) calculated that coefficient of inheritance for the number of leaves was 67 %. Shyu et al. (1975) studied the heritability in flue-cured tobacco and estimated various heritability values for different crosses.

They reported that heritability estimates for the number of leaves were over 84 %.. Studies on tobaccos originating from the Dupnitza region showed high inheritance rate of the number of leaves and it opens a possibility for selecting elite plants with greater number of leaves (Stankev, 2001). In other studies,, high values of inheritance coefficient (in the broad sense) for the number of leaves was recorded, providing the opportunity for fast stabilization in the following generations (Palakartcheva and Yancheva, 1985; Stankev, 1988).

The aim of our investigation was to determine, through hybridological analysis, the character and extent of gene interactions, the number of genes distinguishing the initial parent forms as well as the occurrence of heterosis and transgression in relation to the character number of leaves, for selection of the desired genotypes in the studied hybrid populations of Virginia tobacco.

MATERIAL AND METHODS

P_1, P_2, F_1 and F_2 were studied in six crosses involving local varieties and Virginia tobacco introduced from the USA: Hybrid 714 (K 730 x K 254), Hybrid 715 (K 730 x K 358), Hybrid 719 (RG 8 x K 358), Hybrid 725 (K340 x K 358), Hybrid 726 (K 358 x NC 729) and Hybrid 727 (K 358 x K 254)

The experimental activities were carried out on the trial field of the ITTP in Markovo. With regard to the number of leaves the following calculations were made: mean arithmetic value (x), error of the arithmetic mean (Sx %), degree of dominance (d/a) using Mather's formula and heterosis effect regarding the better parent form (HP). Sobolev's method (8) was employed to determine the following: transgression index (Tп), number of genes differentiating between the parent forms (N), dominancy (D), epistasis (E), character inheritability coefficient (H^2), effectiveness coefficient of the genotype selection using the phenotype expression of the specific character (Pp).

RESULTS AND DISCUSSION

The data in Table 1 show that the number of leaves per plant was inherited overdominantly in all crosses where the parents possessing higher values of the studied trait were predominant,

respectively K 730 and K358. The heterosis effect with economic importance was established in the following combinations: Hybrid 726 (111%), Hybrid 714 –(10%) and Hybrid 719 – (9%). Hetorosis can be used as a method for increasing the number of leaves in Virginia tobacco.

Table 1. Biometrical data on the number of leaves per plant

Parents/crosses	P_1 x±Sx%	P_2 x±Sx%	F_1 x±Sx%	F_2 x±Sx%	d/a	HP
Hybrid 714 (K 730 x K 254)	25,2±0,63	24,2±0,68	27,7±0,54	28,1±1,04	6	109,9
Hybrid 715 (K 730 x K 358)	25,2±0,63	27,2±0,70	28,2±0,98	28,5±0,90	1,3	103,7
Hybrid 719 (RG 8 x K 358)	26,5±0,89	27,2±0,70	28,9±0,58	29,4±0,81	1,7	106,3
Hybrid 725 (K340 x K 358)	26,0±0,78	27,2±0,70	29,6±0,91	29,7±0,87	2,4	109
Hybrid 726 (K 358 x NC 729)	27,2±0,70	26,7±0,85	30,2±0,99	30,8±0,95	13	111
Hybrid 727 (K 358 x K 254)	27,2±0,70	24,2±0,68	27,5±0,82	28,7±1,01	1,2	101,1

The data from the hybridological analysis (Table 2) show that the number of genes controlling the studied character and differentiating between the parent forms varies from 1 to 12 (Hybrid 714).

The values of the transgression index point to the fact that in the process of generation disintegration of Hybrid 714, Hybrid 725 and Hybrid 726, it was possible to select plants over-numbering the parent leaves by 1 to 2 from the available homozygotic phenotypes. In the cross Hybrid 719, forms possessing a leaf-number close to the parents' would prevail and in Hybrid 715 and Hybrid 727 – forms possessing a smaller number.

The strongly pronounced positive epistasis reinforced the phenotype expression of the character number of leaves.per plant The genetic share in the general phenotypic expression of leaf-number in the most of the studied combinations was relatively high and is above 50 % – an indication for starting the selection in the sooner hybrid generations. Only with Hybrid 725 the selection may start in the later hybrid generations.

Table 2. Genetic characteristics of the leaf number per plant

Crosses	$T_п$	N	D	E	H^2	Pp
Hybrid 714 (K 730 x K 254)	1,59	11,77	11,22	22,28	-0,53	18,43
Hybrid 715 (K 730 x K 358)	0,51	8,48	8,52	19,48	-0,47	15,19
Hybrid 719 (RG 8 x K 358)	0,96	7,05	-12,16	28,33	-0,59	17,54
Hybrid 725 (K340 x K 358)	1,35	-9,14	-8,73	33,80	-0,36	14,17
Hybrid 726 (K 358 x NC 729)	1,87	11,16	9,46	19,66	-0,57	19,25
Hybrid 727 (K 358 x K 254)	0,44	-5,62	-14,63	25,44	-0,63	23,28

CONCLUSIONS

1. The character number of leaves in Virginia tobacco crosses is inherited overdominantly, always in direction of the parent displaying a higher value of the character.

2. The number of genes influencing the expression of the studied character and differentiating between the parent components varies from 1 to 12. The strongly pronounced positive epistasis reinforces the phenotype expression of the character number of leaves per plant.

3. Significant positive heterosis was observed in 3 of the crosses, which confirms the heterosis as perspective for the character number of leaves per plant in Virginia tobacco.

4. The high values obtained for the inheritance coefficient and for effective mass selection of phenotypes show that the selection of genotypes characterized by higher number of leaves will be more effective in the sooner hybrid generations.

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INFLUENCE OF SOME SOIL PARAMETERS ON Cu AND Zn CONTENTS IN THE ORIENTAL TOBACOO

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ABSTRACT

Concentration of metals in soils is associated with biological and geochemical cycles as well as with the anthropogenic influence, such as agricultural and industrial activities, transport, and waste management. Tobacco can easily accumulate heavy metals in leaves. The objectives of this study were to determine Cu and Zn concentrations in tobacco leaves produced in different growing areas in Macedonia as well as to determine the most important soil parameters (chemical and physical), which influence the concentration of the above-mentioned metals in Oriental tobacco leaves.

The concentration of heavy metals in tobacco is influenced by many factors, such as: soil type, reaction pH, stalk position, application of metal containing pesticides, soil treatment, fertilization and priming. For that purpose, 50 soil samples were taken from different locations in Macedonia, i.e. family farms that grow Oriental tobacco. A correlation was made between soil parameters and concentration of Cu and Zn in the lower, middle and upper primings of tobacco stalk. The study showed a strong correlation between concentrations of the two investigated metals in these three primings. It also showed that the accumulation of these metals in tobacco leaves was not influenced by their content in soil. The Cu and Zn concentrations in the investigated soils and in tobacco were below permissible limit.

Key words: Cu, Zn, correlation, soil parameters, tobacco

ВЛИЈАНИЕ НА НЕКОИ ПОЧВЕНИ ПАРАМЕТРИ ВРЗ СОДРЖИНАТА НА Cu И Zn ВО ОРИЕНТАЛСКИОТ ТУТУН

Концентрациите на металите во почвата се поврзани со геохемиските и биолошките, циклуси, како и антропогените влијанија, пред сé индустриските и земјоделските активности, транспортот и управувањето со отпадот. Тутунот лесно може да акумулира некои тешки метали во своите листови. Цели на оваа студија беа одредувања на концентрациите на бакар и цинк во листовите ориенталски тутун произведен во различни производни реони од Македонија, како и утврдување на влијанието на поважните почвени (хемиски и физички) параметри врз концентрацијата на истите. Содржината на тешките метали во тутунот е под влијание на многу фактори меѓу кои: типот на почвата, почвената реакција, примената на пестициди кои содржат метали, како и од третирањето на почвата, вклучувајќи ги ѓубриња та и бербениот појас. За реализација на поставените цели беа земени 50 почвени примероци од различни локации во Македонија, семејни фарми кои одгледуваат ориенталски тутун. Направена е корелација меѓу почвените параметри и вкупната концентрација на Cu и Zn од трите бербени појаси (долен, среден и горен) на тутунското стебло. Добиените резултати покажуваат дека постои

силна корелација меѓу концентрациите на двата испитувани метали во трите појаси и дека акумулацијата на овие метали во тутунските листови не е под влијание на нивната содржина во почвата. Концентрација на Cu и Zn во испитуваните почви и тутун е под дозволените граници.

Клучни зборови: Cu, Zn, корелација, почвени параметри, тутун

INTRODUCTION

In the group of agricultural products, tobacco plays a significant role in the agricultural production of the Republic of Macedonia. The export of agricultural products, besides wine, grapes, fruit and some early garden crops, consists mostly of tobacco and tobacco products. The specific climate and soil conditions in Macedonia are suitable for growing Oriental tobacco.

Specific physicochemical characteristics make the Macedonian tobacco an irreplaceable component of the blend used in large number of the World's famous cigarettes brands . Copper and zinc concentrations in soil are affected by a large number of processes contributing to their released amounts, transport, and creating different complex compounds. This study shows the correlation between certain basic soil characteristics and the overall Cu and Zn concentration in the three primings of the Oriental tobacco grown in various parts of Macedonia.

It is well known that micronutrients such as iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) are essential metals for plant growth and yield. Higher concentrations of these elements can infiltrate into the food chain, becoming increasingly dangerous **to** humans and wildlife. Zn and Cu are enzyme activators. Application of fungicides containing zinc and some mineral waste can also increase the concentration of zinc in soils. Besides Zn, Cu is also an enzyme activator and is involved in chlorophyll formation (Tucker, 1999)**.**

Soil reaction is one of the major factors influencing the metal concentration in tobacco leaves (Adamu, 1989, Sanders et al. 1986; Anderson et al. 1988, Golia et al.2001, Zaprijanova et al, 2010). A significant negative correlation between pH of soil and heavy metal content in oriental tobacco was determined in Golia's studies et al. 2007. Husnjak et al., 2009, stated the same, indicating that heavy metal content in tobacco is influenced individually, or interactively by several parameters such as soil reaction (pH), organic matter content (humus), mechanical content (percentage of clay), etc. Adamu et al., 1989, and Zaprijanova et al., 2010, found that humus content influences the heavy metal concentration, especially that of Pb and Cd.

MATERIALS AND METHODS

50 composite soil samples were collected from pedological profiles at fixed depths of; 0 - 10 cm, 10 – 20 and 20-30 cm. Two samples from each locality were taken during November, 2010. The same localities were also used for sampling dry tobacco from the lower, middle and upper primings. The lower primings included the sand and bottom leaves. The middle primings included the first, second and third middle leaves. In the upper primings were lower top and top leaves. Samples were taken from family farms in the well-known tobacco-growing regions in Macedonia (Prilep, Krivogashtani, Mogila, Novaci, Bitola, Demir Hisar, Krushevo, Dolneni, Veles, Cashka, Studenicani) and in some regions of Eastern Macedonia (Strumica, Vasilevo, Bosilevo, Novo Selo, Radovish and Konce). Sampling was made in accordance with ISO 11464:2006. First they were air-dried, and after that crushed and sieved through a 2-mm sieve. Physical properties were determined such as; clay content (Korunović & S.V. Stojanović, 1989), pH (10390:2005), total nitrogen (modified Kjeldahl method-ISO 11361:1995), humus (standard method developed by I.V. Tjurin, modified by Simakov), available phosphorus and potassium (Al-method, validated at the Scientific Tobacco Institute - Prilep, Macedonia, 2009). The total concentration of metals was determined using the Aqua Regia (HCl-HNO_3, 3:1) extraction

method (ISO 11047:1998) after digestion at 180ºC for 2 h. All reagents were of analytical grade (Merck, Germany). Appropriate blanks were included in all extractions. The data were statistically analyzed using correlation analysis (Pearson correlation, two-tailed). Results from two replicates were averaged prior to statistical analyses. Statistical analyses were performed using the SPSS 9.0 software. Correlation analysis was used to establish a relationship between physical and chemical characteristics of soil samples and the heavy metals content in the Oriental tobacco leaf samples from the three positions in the plant.

RESULTS AND DISCUSSION

The analysis of soil composition shows a high variation of the major physical and chemical properties which define soil fertility (Table 1). The clay content varied from 19.50 to 77.6% and pH ranged from 6.00-8.50. According to the results, 54% of the samples showed low humus content, 42% were with average content and 4% with very low and good content. The total nitrogen content was low and similar to that of the humus. The reaction of soil (pH) was neutral in 58% of the soils, 16% were weakly acid and 20% of the samples had poorly to moderately alkaline reaction. 80% of the soils were non-carbonate, 6% were poorly carbonate, 8% moderately and 6% strongly carbonate. 36% of the samples had low and extremely low concentration of phosphorus. The available phosphorus and potassium concentrations varied from 1.54 to 17.96 mg/100g and 3.18- 22.96 mg/100g soil, respectively.

Table 1. Basic soil properties, descriptive statistics

Statistical index	Humus %	Total Nitrogen %	Soil reaction (pH)		Clay %	mg/100 g soil	
			H_2O	(pH) KCl		P_20_5	K_2O
Mean	1.64	0.08	6.97	5.82	39.41	17.96	22.96
Minimum	0.77	0.01	6.00	4.84	19.50	1.54	3.18
Maximum	3.21	0.40	8.50	7.23	77.60	73.77	60.93
CV, %	33.14	66.92	8.63	10.29	31.50	102.66	34.66

The Cu content in the examined soils ranges from 1 to 20 mg/kg . Zn concentration varied from 2 to 34.10 mg/kg (Table 2).

Table 2. Content of Cu and Zn in tobacco and soil (n=50)

Elements	Statistical index	Tobacco			Soil Depth (cm)		
		Lower primings	Middle primings	Upper primings	0-10	10-20	20-30
Cu mg/kg	Mean	5.25	6.12	5.46	17.69	17.75	17.81
	Minimum	1.95	1.35	1.90	5.10	6.17	6.33
	Maximum	15.85	13.00	10.25	45.07	44.27	41.57
	CV, %	51.24	47.86	36.88	50.47	50.84	48.73
Zn mg/kg	Mean	13.67	20.98	18.51	43.24	46.04	49.60
	Minimum	2.19	4.58	6.37	15.53	15.68	17.53
	Maximum	34.10	210.50	185.25	81.76	117.61	127.75
	CV, %	44.22	136.60	134.78	40.19	49.03	44.68

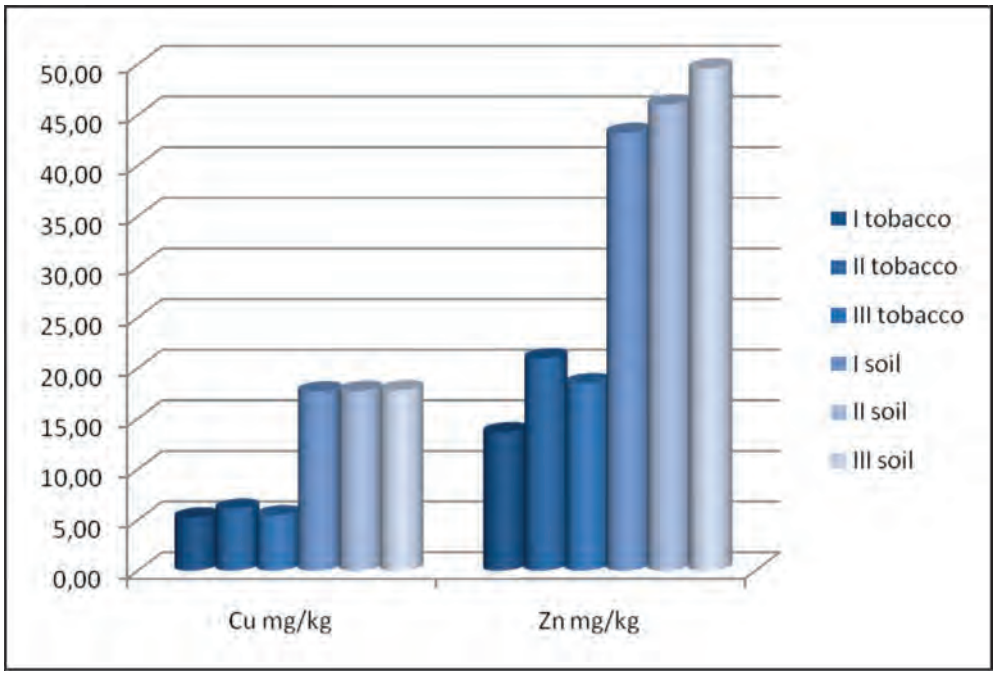

Figure 1. Average concentrations of Cu and Zn in dry soils and tobacco

The average concentrations od Cu and Zn in tobacco and soil samples (Figure 1) indicate that soil has higher concentrations of metal compared to tobacco. Both elements had higer concentrations in the second priming of raw tobacco samples. Cu content in soil had a steady distribution in all three layers (0-10, 10-20 and 20-30 cm), while Zn concentration was higher in the IInd and the IIIrd layer.

Table 3. Correlation between soil parameters and the concentration of Cu and Zn in Oriental tobacco (n = 50)

Parameter	Cu 1st	Cu 2nd	Cu 3^{d}	Zn 1st	Zn 2nd	Zn 3rd
Clay %	0.279*	0.026	0.001	0.323*	0.173	-0.212
pH	0.039	0.047	0.112	-0.060	0.079	-0.140
Cu 1st		0.540*	-0.041**	0.153	0.191	-0.059
Cu 2nd			0.497**	0.202	0.137	0.211
Cu 3^{d}				0.003	0.109	0.126
Zn1st					-0.050	-0.036
Zn 2nd						0.091
Zn 3^{d}						

1st - lower primings 2nd – middle primings 3rd – upper primings

*.Correlation is significant at 0.05 level (2-tailed).
**.Correlation is significant at 0.01 level (2-tailed)

Descriptive statistics for Cu and Zn contents in soils and tobacco leaves from the studied area is presented in Table 2. Higher concentrations of Zn and Cu were recorded in the second primings. Correlation coefficients between total metal concentrations in tobacco and soil parameters (Table 3) showed a strong relationship among the concentrations of each

tobacco priming (lower, middle and upper). Cu had a significant correlation at 0.01 level, which was not the case with Zn. Clay had a poor influence (correlation is significant at 0.05 level) on the concentration of Cu, and Zn in the first priming of Oriental tobacco. No significant correlation was noticed between reaction pH and metal contents in our investigations, which does not coincide with those of some authors, who reported strong correlation (Adamu et al., 1989; Golia et al. 2007, Zapryanova, 2010).

CONCLUSION

According to the results, it can be stated that most of the soils are ideal for producing high quality Oriental tobacco. Concentrations of Cu and Zn in investigated tobacco plants and soils in R. Macedonia are below permissible limit values in conventional and ecological agriculture. Comparing the results of the investigation, it can be concluded that heavy metal content in soil has a negligible or no influence upon the heavy metal content in tobacco.

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THE INFLUENCE OF SECONDARY TILLAGE ON SOIL COMPACTION AND THE YIELD OF FLUE CURED TOBACCO

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ABSTRACT

During 2005 and 2006 a research has been carried out on the effect that application of chisel had on the flue-cured tobacco production. The research has been carried out on luvisol in the field experiment performed on the experimental field plot of Tobacco Institute of Zagreb at Pitomača. In addition to the classical tillage practices (ploughing in the autumn + soil preparation in the spring) during the spring tillage and tobacco planting preparation chisel was used. During the vegetation period, the soil was hoed up manually (I), loosened with the cultivator (II) and with chisel between tobacco rows (III). The electronic penetrometer was used for measurement of soil resistance during tobacco flowering period. The tobacco was picked six times and after the lust harvesting and flue-curing the leaf yield was determined. All data wepe processed with the statistical variance analysis. The soil resistance was lower and the tobacco yield was higher where soil was tilled with chisel as compared to the conventional soil tillage practices in the tobacco production.

Key words: tobacco, soil tillage, bulk density, tobacco yield.

ВЛИЈАНИЕ НА ДОПОЛНИТЕЛНАТА ОБРАБОТКА ВРЗ ЗБИЕНОСТА НА ПОЧВАТА И ПРИНОСОТ НА ТУТУН ОД ТИПОТ ВИРЏИНИЈА

Во текот на 2005 и 2006 година беше извршено истражување на ефектот од примената на секундарната обработка на почвата врз типот вирџинија. Истражувањето е извршено на лувисол, на експерименталните површини од Институтот за тутун - Загреб во Питомача. Покрај класичната обработка на почвата (орање во есен и подготовки на почвата во пролет), во текот на пролетната обработка и подготовките за садење на тутунот, применет е подривач. Во текот на вегетационият период, почвата беше рачно обработена (I), растресена со култиватор (II), и со подривач меѓу редовите(III). Електронски пенетрометар беше користен за мерење на почвениот отпор за време на цветањето на тутунот. Тутунот беше берен на 6 пати, а по последната берба беше утврден приносот на тутун. Сите резултати се статистички обработени со анализа на варијансата. Отпорот на збиеност на почвата беше помал, а приносот на тутунски лист повисок онаму каде што почвата беше обработена со подривач, во споредба со вообичаените мерки на почвена обработка во производството на тутун.

Клучни зборови: тутун, обработка на почвата, збиеност на почвата, принос на тутун.

INTRODUCTION

Virginia type tobacco was introduced in Croatia in the 50's for the needs of tobacco production. Initially, it was planted on sandy soils and later the production spread to heavier soils in Northern Croatia, from Đurđevac to Donji Miholjac. Economic effects and the size of farms are the main reasons why tobacco is often grown as a monoculture or in a very narrow crop rotation. Perennial tobacco growing in a monoculture on soils prone to compaction without adding organic matter led to degradation of arable layer, what was the reason of lower yield. Often shallow soil tillage on the same depth led to increased compaction in subploughing layer, especially on ploughing depth.

The major problems of tobacco soils in Croatia for current state of arable layer are of generic origin and the consequences of irregular managing of these soils. Physical characteristics of these soils are often deteriorated to the extent that water occasionally retain on the soil surface. Often emphasized compaction of plough and subplough layer makes the growth and development of the root system harder and it's the main limiting factor for achieving higher yield and better leaf quality (Turšić, I., 1994).

Yield and quality of tobacco, with the genetic potential of cultivar, significantly depend on the current soil fertility, applied agricultural practices and climatic conditions during the vegetation period (Akehurst, 1981, Hawks, Collins, 1983). Approach to managing the soil-plant system in the growing area of Virginia tobacco has to include measures such as crop rotation, calcification, appropriate fertilizer application, soil tillage, erosion, control and conservation of water in soil. Not one measure can replace the other or profitable production can be maintained until all factors which affect the productivity are well balanced.

MATERIAL AND METHODS

The influence of secondary tillage on yield of tobacco and soil compaction was investigated on experimental field in Podravina. The experiments were carried out on field trial on luvisol in four repeats according to split block method (Vasilj, 1974).

Half of experimental field (528m^2) was loosened with chisel (Table 1). On the other half disc plough was applied and then cultivator. The trial had six combinations (2×3). There were four rows of tobacco per repetition and measurements were taken on two rows in the middle.

Table 1. Trial procedures

Soil preparation for tobacco planting	Soil cultivation during the vegetation period
1. Chisel Cultivator	1.1. Manual cultivation 1.2. Cultivator 1.3. Chisel + cultivator
2. Disc plough Cultivator	2.1. Manual cultivation 2.2. Cultivator 2.3. Chisel + cultivator

The length of test field was 10m and the spacing between rows was 1.1m. The area of the main field was 44m^2. In total 528m^2 was loosened in spring (44m^2 × 3 procedures × 4 repetitions). The chisel wasn't applied on the remaining 528 m^2 but the soil was disced before the application of cultivator and tobacco planting. In the tobacco flowering (at the end of July) the soil resistance (compaction) was measured and at the same time the samples for soil moisture were taken. Soil resistance was measured by electronic penetrometer (Košutić. 1989). Current soil moisture in plough and subplough layer is determined gravimetrically.

RESULTS AND DISCUSSION

The soil on experimental field was sandy loam with high content of fine sand and dust and low content of clay, with unstable structure and tend to compaction and crust formation (Table 2).

Table 2. Mechanical composition of soil from experimental field

Soil horizon	Depth (cm)	Percentage of particles				Texture
		Coarse sand (2-0.2 µm)	Fine sand (0.2-0.02 µm)	Silt (0.02-0.002 µm)	Clay (<0.002 µm)	
A_p	0-26	15	58	17	10	sandy loam
E	26-45	18	61	15	6	sandy loam
B_i	45-90	21	36	22	21	loam

Mechanical composition and other physical properties were shown in Table 3.

Table 3. Physical properties of the soil

Soil horizon	Total porosity (%)	Field water capacity (%)	Air capacity (%)	Bulk density	
				Stv	Stp
A_p	45.6	36.4	9.2	1.46	2.70
E	38.3	33.7	4.6	1.68	2.72
B_t	41.6	34.1	7.5	1.71	2.74

Air capacity is moderately low in ploughing and iluvial horizon. Field water capacity is average in ploughing and iluvial horizon and very low in eluvial horizon. According to bulk density values soil compaction of subploughing layers is significantly higher than compaction of ploughing layer.

The application of chisel on soil of stated physical characteristics was found as fully justified. The procedure in which the chisel was applied in spring during the soil preparation, a significantly lower resistance was measured (figure 1), which resulted in significantly larger yield of tobacco leaf (Soane, Ouwerkerk, 1994, Turšić, 1992).

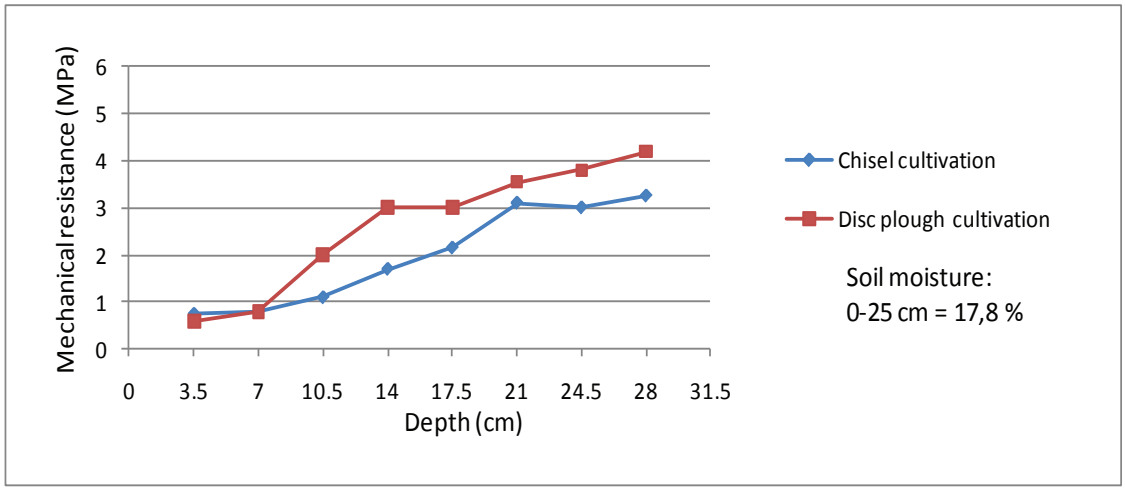

Figure 1. Influence of chisel plough tillage on soil compaction

In procedure in which the chisel was not applied a larger soil resistance was determined. Increased soil compaction conditioned by texture and the way of soil preparation reduced the growth and development of root system into the deeper layers and decreased the tobacco yield (Turšić et al. 1994).

Table 4. Influence of chisel application in soil preparation on tobacco yield, Pitomača

Soil tillage	Yield, kg/ha		$\overline{X}$
	2005	2006	
Chisel	2667,0	2733,3	2700,15
Without chisel	2336,3	2499,3	2417,00
LSD, 5%	131,2	210,1	

Lower soil resistances (lower compaction) were measured in both years of research where during the vegetation period the soil was loosened with the chisel between the rows.

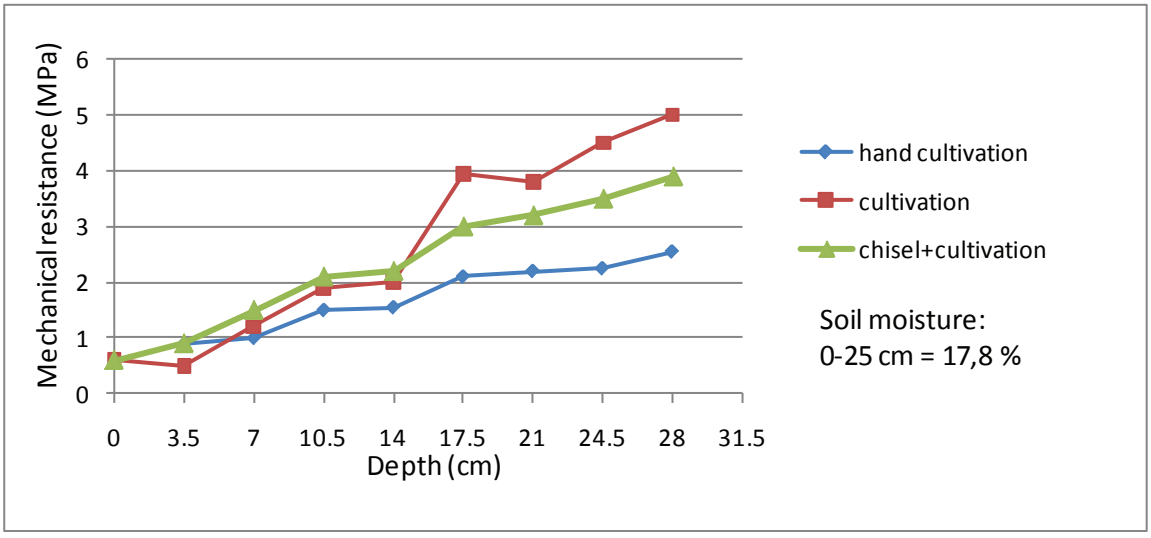

Figure 2. Influence of interrow chisel plough tillage on soil compaction

It is known that the largest part of the tobacco root system develops at depth of 30 to 40 cm (Turšić, 1989, 1994, Vepraskas et al.1986). First two weeks after transplantation tobacco develops lateral roots and that is the reason why first soil tillage between rows is conducted deeper, with the machines similar to the chisel applied in this experiment (Akehurst, 1981).

As the tobacco plants become higher and the root system develops deeper, the next cultivations are conducted shallower and their main goal is soil ventilation (crust breaking), weed removal and drainage of excess water. During the procedure in which chisel was applied between the rows, the soil was less compacted and the larger tobacco yield was obtained (Table 5).

Table 5. Influence of interrow chisel plough tillage on flue-cured tobacco yield, Pitomača 2005-2006

Interrow tillage	Yield, kg/ha		$\overline{X}$
	2005	2006	
Manual cultivation	2393,0	2649	2521
Cultivator	2422,0	2530	2476
Chisel + cultivator	2690,0	2780	2735
LSD, 5%	215,3	196,0	

Soil loosening between rows has significantly increased the tobacco yield (McKee, 1988). In average chisel application in first cultivation has increased the tobacco yield by 8,31-11,05 %.

CONCLUSIONS

The investigation of diferent types of soil preparation for tobacco cultivation has shown that chiesel application has significant advantage compared to other types of cultivation.

The application of disc plough in secondary cultivation has increased soil compaction and decreased yield of tobacco leaf so it should be avoided in soil preparation.

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Original scientific paper

THE USE OF GLYPHOSATE (N-(PHOSPHONOMETHYL) GLYCINE) IN SUCKER CONTROL OF TOBACCO

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ABSTRACT

Topping of tobacco (cutting off the floral buds) is cultural practice which guarantees a high economic effect reflected in increased yield and quality. In normal conditions of growing, the topping is accompanied by vigorous formation of suckers. Removal of floral buds and suckers can be performed in two ways: mechanically (by hand or with specialized technique) and chemical (with application of physiologically active substances - FAS). According to the type of their effect on suckers, FAS are divided in those with contact activity and those with systemic activity. So far, from the products with systemic activity the salts (potassium or hallein) of the maleic hydrazide (MH) have been used. After the deadline for its use in Bulgaria has expired, there are no new systemic products to substitute it. The aim of investigation was to study the possibilities for application of the herbicide Glyphosate not only for chemical topping of tobacco plants but also for control of suckers appearing after topping, with high technical and economical effectiveness among parasitic formations, free of phytotoxic effect on the crop.

Key words: tobacco, topping, sucker formation, sucker control, active substances

УПОТРЕБАТА НА ГЛИФОСАТ (Н-(ФОСФОНОМЕТИЛ) ГЛИЦИН) ПРИ КОНТРОЛАТА НА ФИЛИЗИТЕ

Поткршувањето на цветни китка е културна традиција која гарантира висок економски ефект со зголемен принос и квалитет. Во нормални услови на растење, на врвот од тутунското растение се појавува силно формирање на филизи. Отстранувањето на цветните китки и филизите се врши на 2 начина: механички (рачно или со специјална техника) и хемиски (со примена на физиолошки активни супстанции – ФАС). Според нивното влијание врз филизите, ФАС се поделени на контактни и системични. До сега, од производите со системична активност се користеа солите на калиум или халлеин и од малеик хидразид (МН). По истекот на рокот за нивната употреба во Бугарија, не постојат други системични препарати кои би ги замениле.

Целта на ова истражување е да се проучат можностите за примена на хербицидот глифосат покрај неговата примена како хербицид и за контрола на филизите. Оваа употреба има висока техничка и економска ефекасност, без фитотоксичен ефект врз растенијата.

Клучни зборови: тутун, отстранување на филизи, контрола на филизи, активни супстанци.

INTRODUCTION

Topping of large-leaf tobaccos is cultural practice which gives high economic effect, reflected in increase of yield and quality. The same is valid for the oriental tobaccos, although in that case it is more difficult to be realised in practice.

Cutting off the floral buds makes presumptions for qualitatively different mechanism of distribution and deposition of assimilates created in tobacco leaves. They will not go mainly to the flower bud, but will be distributed in leaves, due to which they become larger, more vigorous and substantial, with higher weight and quality.

In normal conditions of tobacco growth, topping is accompanied by increased suckering. The newly established influx of anti-growth hormones formed in the top part of tobacco plant with the purpose to prevent the growth of side buds on the stem under leaf base, after cutting off the top becomes the reason for intensive sucker formation (6). The suckers are divided into: primary - highly visible, secondary - poorly visible at leaf base and thirdly - existing, but not visible. From the economic point of view, suckers can be considered as parasitic formations which draw considerable amount of assimilates from the plant for their growth (1).

Removal of floral buds, as well as of the suckers, can be performed in two ways: mechanically (by hand, with specialized technique) and chemically (with application of FAS).

Manual sucker control is labor-consuming technique with a short-term effect. By application of FAS, a better and long-lasting effect is achieved.

According to the type of their effect on suckers, FAS are divided in those with contact activity and those with systemic activity (6).

The chemical with contact activity used on large-leaf tobaccos in Bulgaria is pendimethalin (Stomp 330 EK).

Under the influence of pendimethalin , suckers embryos in leaf base rapidly turn to yellow, brown, and then die. However, 10 -12 days after the occurrence of secondary suckers is observed and they grow fast if plants have not been treated with the same or with some other systemic chemical. So far, contact chemicals in our country have been applied only manually.

Until recently, from the products with systemic activity the salts (potassium or hallein) of the maleic hydrazide (MH) have been used. They are absorbed form the leaves of treated tobacco plants and transported to the growing tissues - suckers, preventing their growth without killing them. Thus, inhibited but still alive suckers do not allow development of secondary buds, leaving the plants free of suckers for a longer period (7 - 8 weeks) (5). Systemic chemicals can be incorporated in the soil or foliary.

In oriental tobacco there is no specialized technique for topping and treatment, although this is highly effective measure for this type, too. Manual topping is also very hard to perform. For this reason, chemical topping of plants finds its place here, implemented with systemic chemicals (MH). The effectiveness of the applied measure is reflected in 20-30% higher yield per decare and in improved quality of tobacco (2).

Until recently, the problem of sucker formation in tobacco was solved by application of the potassium salt of maleic hydrazide (Royal MH-3). After the deadline for its use in Bulgaria has expired (18.07.2009), there are no new systemic chemicals to substitute it. Maleic hydrazide is essentually a herbicide, which gave us an idea to search for a product with similar effect as the herbicides already known in practice.

The mechanism of action of glyphosate (N-(phosphonomethyl) glycine) has been known for a long time. It is a total herbicide with systemic action. The herbicide is absorbed by tobacco leaves and transferred toward the growing (meristem) parts of the plant - : root, top and suckers (3). When glyphosate is applied by spraying of the top parts of the plant, significant part of the herbicide is accumulated in the nearby (meristem) tissues - vegetative top, leaves of the vegetative top and suckers. No published data can be found in Bulgarian and world literature on application of glyphosate for chemical topping or sucker control in tobacco.

The aim of investigation was to study

the possibilities for application of the herbicide Glyphosate both for chemical topping of tobacco plants and for control of suckers appearing after topping, with high technical and economical effectiveness among parasitic formations and no phototoxic effects on the crop.

MATERIAL AND METHODS

Two-year investigations (2009 - 2010) were carried out in the experimental field of Tobacco and Tobacco products Institute in Markovo. Tobacco plants were treated with the herbicide Glyphogan 480 SL (glyphosate/n-(phosphonomethyl)- glycine, isopropylamine salt) of Agan Chemical Manufacturers Ltd, and Royal MH-30 (21.7% maleic hydrazide, potassium salt) of the company Crompton Uniroyal Chemical was used as a standard.

In the first investigation year (2009) the task of the trial was to estimate the possibility of glyphosate application in sucker control and to determine the effective rate in oriental tobacco Basma (variety Plovdiv 7) and the large-leaf Virginia (variety Virginia 0514). Three rates were investigated in the oriental variety (50, 100 and 200 ml/dca) and three in Virginia (100, 200 and 300 ml/dca).

During the second year of investigation (2010), the trial was set up with Virginia 0514, to compare technical effectiveness of glyphosate with the standard maleic hydrazide and investigation of possibilities for chemical topping of tobacco inflorescences. Glyphosate (Glyphogan 480 SL) was applied in a rate of 300 ml/dca, and the standard maleic hydrazide (Royal MH-30) in a rate of 1500 ml/dca. Topped but untreated tobacco was used as a check.

Trials were set up by the method of long plots with four replications. The size of the plots was 10 m^2 and 20 m^2 in the first and the second investigation years respectively. Tobacco plants were topped up to the 20-th leaf, in the beginning of flowering stage (10-15% of plants are flowered in the plots treated immediately before topping, applying a back sprayer with 30 l/dca solution. For chemical topping of the inflorescences, tobacco was treated in the stage of buttonization, with Glyphogan 480 SL in a rate of 300 ml/dca. Plants were monitored 10 and 20 days after topping and treatment. Readings were made on suckers type and growth as well as on the occurrence of phytotoxic effects on tobacco (according to EWRS scale).

RESULTS AND DISCUSSION

Results of investigations in 2009 are presented in Table 1.

In oriental tobaccos, after removal of floral buds and plant treatment, suckers growth recorded on the 10th day was respectively 6-8 cm with solution rate of 50 ml/dca, 4-6 cm in a rate of 100 ml/dca, 2-4 ml in a rate of 200 ml/dca and 6-8 cm in the topped but untreated plants. On the 20th day of topping and treatment, the readings for suckers growth were as follows: 10-15 cm for the lower rate, 8-10 cm for the medium and 4-6 cm for the higher rate.

In large-leaf tobaccos, suckers growth recorded on the 10th day from removal of floral buds and plant treatment was respectively 8-10 cm with solution rate of 100 ml/dca, 6-8 cm in a rate of 200 ml/dca, 3-5 ml in a rate of 300 ml/dca and 12-15 cm in the untreated check variant. As can be seen from the results in both tobacco types, low herbicide rates (50 - 100 ml/dca) fail to achieve effective control of suckers and their growth is similar to that of the check variant. High herbicide rates (200-300 ml/dca) give good control of suckers, with phytotoxic effects typical for the glyphosate - etiolation, prevention of their growth and occurrence of necrosis. In the same time, none of the investigated rates has shown phytotoxic effect on treated tobacco plants.

Tables 2 and Photo 1 and 2 present the results of the glyphosate and the standard Royal MH-30 effects on the suckers 20 days after topping and treatment of tobacco plants. Both

the physiological effects and the effectiveness of glyphosate and maleic hydrazide are similar. In contrast to the standard, however, the effective rate of glyphosate is 5 times lower, which supposes lower value of the treatment. Duration of the effects of both chemicals on suckers control is 20 days after treatment.

Suckers in the check plants (topped but untreated) are developing freely.

Table 1. Results of investigation of Glyphosate on suckers control in 2009

Variants Tobacco type Rate (ml/dca)	Time of monitoring, suckers growth (cm), phytotoxicity on tobacco		
	10th day	20th day	EWRS
Oriental tobacco			
50	6-8	10-15	0
100	4-6	8-10	0
200	2-4	4-6	0
Check	6-8	12-15	-
Large-leaf tobacco			
100	8-10	15-20	0
200	6-8	10-15	0
300	2-4	3-5	0
Check	12-15	20-22	-

Table 2. The effectiveness of Glyphogan 480 SL application on sucker formation in 2010

Chemicals	Rate (Conc.)	Suckers growth and size				Effective-ness %	Duration of the effect (in days)
		I reading	II read.	III read.	N		
Glyphogan 480 SL	300 ml/ dca	1-2	No 1-2 cm	No 1-2 cm		100	20
Royal MH-30 (standard)	1500 ml/dca	1-2	No 1-2 cm	No 1-2 cm		100	20
Check	-	1-2	Free 7-8 cm	Free 12-14 cm		-	-

Excellent results were also obtained in the trial when glyphosate was applied for chemical treatment of tobacco. Photo 5 shows the effect on plant 10 days after treatment of tobacco bud.

Photo 1 Tobacco topped and treated with Glyphogan 480 SL with 300ml/dca - 20 days after treatment

Photo 2 Tobacco topped and treated with Royal MH-30 with 1500ml/dca - 20 days after treatment

Photo 3 Topped, untreated tobacco - 20 days after treatment

Photo 4 Tobacco plant prior to treatment of floral bud for chemical topping

Photo 5 Tobacco plant 10 days after treatment of floral bud with Glyphogan 480 SL - 300 ml/dca

Observations were made on phototoxicity in the top 4-5 leaves , together with necrosis and falling out of floral buds. Plants which have completed their growth at 22nd-23rd leaf are not affected by the herbicide and no symptoms of phytotoxicity are observed on them. In manual or mechanical topping the affected leaves are removed together with flowers, so that they have no reflection upon the yield. In addition to this, despite the chemical topping of floral bud, no suckers development is observed on plants. Chemical topping of tobacco plants will result in higher productional and economic effect - reduction of manual labor with simultaneous removal (topping) of floral buds and sucker control in the plants.

CONCLUSIONS

1. For the first time possibilities were made for application of glyphosate as physiologically active substance in both chemical topping and sucker control in tobacco.

2. Physiological effect of glyphosate is identical with that of the maleic hydrazide which has been used with the same purpose so far.

3. No symptoms of negative phytotoxic effect have been observed in treatments with recommended rates of glyphosate.

4. Chemical topping of tobacco plants with glyphosate yields higher productional and economic effect - reduced manual labor with simultaneous removal (topping) of floral buds and sucker control in the plants.

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TOBACCO ETCH VIRUS-TEV ON TOBACCO IN BULGARIA

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ABSTRACT

This paper presents scientific communication on the presence of Tobacco etch virus (TEV) on tobacco in Bulgaria.

Immunological investigations were carried out in 2011 to identify the presence of TEV on tobacco in Bulgaria. Samples were taken from 12 tobacco plants (5 of Virginia tobacco and 7 of the type Burley) from the trial field of Tobacco and Tobacco products Institute Plovdiv (Markovo). ELISA test was performed on the isolates for serological diagnostification of PVY and TEV viruses, which give similar symptoms in tobacco plants

Three isolates of the Virginia tobacco showed positive serological results on both PVY and TEV viruses. One isolate of the Virginia type showed (+)(-) reaction to TEV and negative reaction to PVY. In one isolate of the Burley type negative reaction to both viruses was recorded.

Elimination of weeds surrounding the tobacco fields and due and timely application of insecticides against aphids is recommended for successful control of this disease.

Key words: Tobacco etch virus (TEV), ELISA-тест, tobacco, virginia, burley

ВИРУС НА ГРАВИРАНИОТ МОЗАИК (*TOBACCO ETCH VIRUS-TEV*) НА ТУТУНОТ ВО БУГАРИЈА

Во овој труд е дадено научно соопштение за присуство на вирусот на гравираниот мозаик на тутунот во Бугарија.

Во текот на 2011 година се изврши имунолошки истражувања за докажување на присуството на вирусот на гравираниот мозаик (TEV) на тутунот во Бугарија. Земени се проби од 12 тутунски растенија, и тоа 5 од типот вирџинија и 7 од типот берлеј, од опитното поле од Институтот за тутун и тутунски производи од Пловдив (с. Марково). На изолатите е извршен ELISA-тест (имунолошки тест) за серолошко дијагностицирање на PVY и TEV вирусот, кои предизвикуваат слични симптоми на тутунските растенија.

Три изолати (проби) од типот вирџинија покажаа позитивни серолошки резултати и на двата вируса, PVY и TEV. Еден изолат од типот вирџинија покажа реакција (+)(-) кон TEV и негативна кон PVY. Еден изолат од типот Берлеј негативно реагира на двата вируси.

Во борбата со ова заболување се препорачува уништување на плевелите околу тутунските површини и навремена борба со инсектициди против лисните вошки.

Клучни зборови: вирус на гравираниот мозаик-TEB, Елиса-тест, PVY, вирџинија, берлеј

INTRODUCTION

Viruses pose a serious threat to tobacco crop and can cause severe damages to tobacco production. Very often, losses caused by them make the tobacco production unprofitable and even impossible.

CORESTA investigations so far have determined 16 viruses attacking tobacco, eight of which are of economic importance: Tobacco mosaic virus – **TMV**, Potato virus Y - **PVY**, Cucumber mosaic virus - **CMV**, Tomato spotted wilt virus - **TSWV,** Tobacco etch virus **–TEV,** Tobacco leaf curly virus - **TLCV**, Tobacco vein mottle virus **– TVMV** and Beet curly top virus **–BCTV.**

Tobacco etch virus (TEV) was reported for the first time in Kentucky, USA, by Valleau and Johnson (9). After that, its symptoms were also reported in Canada, Nicaragua, India, Japan, Russia and Germany.

According to Atanasov and Gabrovska (1), the virus has not been recorded on tobacco in Bulgaria. Kovacevski et al. (3) reported occurrence of the disease on tomato and jimson weed (Datura Stramonium L.), but not on tobacco. According to Mickovski (4), the disease is not spread on tobaccos grown in the Balkan Peninsula.

Various methods were used for determination of the causing agents of diseases according to various parameters: disease symptoms (symptomatic diagnostics); the type of cell elements specific for each virus (microscopically determined); the specific reaction for virus identification with indicatory plants, by immunity test (ELISA – serological identification) or through by molecular techniques with radioisotopic markers.

The presence of Tobacco etch mosaic virus (TEV) on the basis of symptomatic diagnostics was reported by Dimitrov and Bozukov in 2004 (2) .

MATERIAL AND METHODS

During 2011 immunological investigations were carried out in order to determine the presence of TEV in Bulgaria. Fresh material (tobacco leaves) with similar symptoms typical for TEV was supplied for the investigation. Selection of isolates for the study was made on the basis of visual and symptomatic determination. Samples were taken from 12 tobacco plants – 5 of the type Virginia and 7 of Burley. Isolates were taken from the trial field of Tobacco and Tobacco Products Institute-Plovdiv (Markovo village).

ELISA test was applied for serological diagnostics of PVY and TEV, which produce similar symptoms on tobacco plants.

The test was performed by the Double Antibody Sandwich-Enzyme Linked Immune Sorbent Assay (DAS ELISA) for PVY and by the Triple Antibody Sandwich-Enzyme Linked Immune Sorbent Assay (TAS ELISA) for TEV, in the laboratories for investigation of immunity to virus diseases at the Institute of Horticulture in Maritza.

RESULTS AND DISCUSSION

Three isolates (samples) of the type Virginia showed positive serological results on both PVY and TEV. One isolate of Virginia tobacco showed (+)(-) reaction to TEV and negative reaction to PVY. One isolate of the type Burley showed negative reaction to both viruses (Table 1).

Table 1. Serological diagnostics of PVY and TEV

Sample №	Tobacco type	TEV	PVY
1	Virginia	+	+
2	Virginia	+	+
3	Virginia	+	+
4	Virginia	+ -	-
5	Virginia	+ -	+
6	Burley	-	+
7	Burley	-	+
8	Burley	-	+
9	Burley	-	+
10	Burley	-	+
11	Burley	-	+
12	Burley	-	+
Negative control	Tobacco	-	-
Positive control	Tobacco	+	+

Tobacco etch virus was serologically proved in mixed virus infection with PVY in three of the test samples.

According to Holmes (7), susceptibility to the virus was found in about 69 plant species from 11 families, some of which (tomato, jimson weed, potato and tobacco) belonging to *Solanaceae* family.

Disease symptoms vary depending on virus strain, type of tobacco and agroecological conditions.

The virus produces variegation of the upper leaves and changes their colour from light green to green. The symptoms sometimes resemble the Tobacco mosaic virus (TMV), but the chlorotic spotting caused by TEV is much smaller than that caused by TMV.

In field conditions, symptoms usually appear when the stage of tobacco flowering is near. The first signs are lightening of the colour of leaf veins, followed by necrotic lines and chlorotic variegation between the veins. In lower primings, the venation can turn brown or disappear. Some authors reported that patterns caused by Tobacco etch virus are sometimes resembling those of Tobacco mosaic virus (TMV), but while in TMV the top leaves are variegated, in TEV they are not.

Depending on their reaction to the virus, tobacco types can be divided into two groups:

- Ist - consisting of Burley tobaccos, which react with strongly pronounced symptoms of chlorosis, necrosis and stunted growth, and
- IInd - consisting of Oriental and Virginia tobaccos, which react only with ocurrence of chlorotic spots.

The virus can be transmitted mechanically and also spread by several species of aphids (Aphis, Myzus etc) (6), or by dodder (Cuscuta sp.). Nikolov et al.(8) reported on virus transmission by seeds of infested tobacco.

Photo 1. Virginia tobacco infected with Tobacco etch virus (TEV)

Virus particles are cylindrical, asymmetrical, approximately 730-790 nanometers long and 10 -13 nanometers wide. The thermal inactivation point of the virus is 55 - 60 ^{o}C. Infection ability is preserved up to 5 days. The virus may persist a dilution of 1:5000 (5), but loses its activity in dry leaves.

The virus enter plants through wounds made by aphids or mechanical injury and the first symptoms appear 7-14 days after the inoculation.

Usually, by infestation of a plant with certain strain of virus, it becomes resistant to other strains serologically related to the same virus, but the infested plants are still susceptible to other viruses. Tobacco etch virus is exception from this rule. Tobacco plants infested with TEV become resistant to Potato virus Y (PVY), too. Contrary to that, tobacco plants infested with PVY do not bear resistance to TEV.

CONCLUSSION

The best control of this disease is to destroy the weeds around tobacco plots, since they are serious source of infection. It should be avoided to grow tobacco near the areas planted with other species of Solanaceae. Since aphids are the main transmitters of the virus, timely treatment with insecticides should be applied.

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MORPHOLOGY AND BIOLOGY OF PARAGUS QUADRIFASCIATUS

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ABSTRACT

P. quadrifasciatus is an obligate aphidophagous species and it has an important role in biological control of aphids in natural agroecosystems. In all years of our investigations it was recorded as predator on *M. persicae* in tobacco fields. This species is among the smallest syrphid flies. The imagos considerably short and dark. The abdomen is black and its first and second segment are also black. The other abdominal segments have visible yellow-white lateral stripes. In laboratory conditions the adults live 6 - 7 days. Females lay their eggs singly on the underside of tobacco leaves, among the aphid colonies. Eggs are ovate, cream-colored, 0.5 - 0.6 mm long. The embryionic development lasts 3 days. Immediately after hatching, larvae are almost colorless to light beige, with denticulate body. Later, the color turns to brown, with expressed thorns on the segments.

Larvae kill a great number of aphids. Their greed increases during the second, and particularly during the third larval stage. Their larval stage average lasts 8 days. Pupae are yellowish-brown and prickly and the duration of pupal stage is about 5-7 days. According to our investigations, the growth of one generation from egg to imago was 16-19 days.

Key words: *Surphid flies, Paragus quadrifasciatus*, *aphids, M. persicae*

МОРФОЛОГИЈА И БИОЛОГИЈА НА PARAGUS QUADRIFASCIATUS

P. quadrifasciatus е облигатен афидофаген вид и има значајна улога во биолошката контрола на лисните вошки во природните агроекосистеми.

Во сите години од нашите испитувања овој вид е утврден како предатор на лисната вошка *M. persicae* на тутунот. Овој вид спаѓа меѓу најмалите осолики муви. Имагата се кратки и со темна боја. Абдоменот е црн. Првиот и вториот стомачен сегмент се исто така црни. На останатите сегменти од стомакот видливи се две поизразени, жолто-бели, попречни пруги. Во лабораториски услови имагата живеат 6-7 дена.Женките ги несат јајцата поединечно, на опачината од тутунските листови меѓу колониите на лисните вошки. Јајцата се овални, со кремова боја, со должина од 0,5 до 0,6 mm. Ембрионалниот развој се одвива за 3 дена.Штотуку испилената ларва е скоро безбојна до светлобежова, назабена по површината на телото. Со текот на развитокот ларвата добива кафена боја, со изразени боцки на сегментите.Ларвите уништуваат голем број на вошки. Лакомоста е зголемена за време на вториот степен, а посебно во третиот ларвен степен. Стадиумот ларва просечно се одвива за 8 дена. Куклите се жолтеникавокафени и боцкави. Стадиумот кукла се одвива за 5 до 7 дена.Според нашите проучувања, развитокот на една генерација од јајце до имаго се одвива во период од 16 до 19 дена.

Клучни зборови: осилки муви *Paragus quadrifasciatus*, лисни вошки *M. persicae*

INTRODUCTION

Paragus quadrifasciatus Meigen, 1822 is an obligate aphidophagous species which develops normally only when fed on aphids.

According to many authors, this species feeds on a wide spectrum of aphids and presents an important factor in biological control of these pests. Januseska (2001)/Krsteska (2007) reported it as a predator of *Myzus persicae* Sulz. on tobacco.

MATERIAL AND METHODS

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

For research of hoverflies in laboratory conditions and for investigation of their biology, standard methodology was applied. Larvae were fed only on *M. persicae.*

Field collected material was analysed in the laboratories of Tobacco Institute on binocular.

Weight of hoverflies in various stages of growth was measured on Sartorius BL 210 S analytical balance (d=0.1 mg), while length and width on Carl Zeiss Jena binocular (25 x 5).

RESULTS AND DISCUSSION

Paragus quadrifasciatus Meigen, 1822

Genus Paragus consists of the smallest hoverflies species.

Imagos of *P. quadrifasciatus* are adapted to thermophilic and xerophilic conditions, which is related to their Mediterranean origin.

During our investigations, imagos were recorded from August 10 to September 1.

P. quadrifasciatus was recorded in the hedges of tobacco fields, near the meadows, vegetable gardens and cereal crops.

According to S i m i c (1987) and G l u m a c (1955), imagos appear from April to August and G a o (1991) reported their appearance from late April, early May and late August to the beginning of September. This species occurs between the aphids in cereal fields, vegetable gardens or in the thick grass.

Females lay their eggs singly on the underside of tobacco leaves, among the aphid colonies.

The eggs are tiny, almost invisible, but as observation progresses, they can be easily seen with naked eye.

Eggs are ovate, cream-colored, 0.5 - 0.6 mm long, with one end rounded and one end sharpened. The outer side of the egg is slightly curved. Eggs are usually deposited horizontally on the leaf. They are seldom found in vertical position, with their micropyle upright.

In our investigations the duration of the egg stage was three days.

According to Gao (1991), the duration of the egg stage at 20^{0}C is three to four days. Ma et al. (1986) also reported 3-4 days duration of the egg stage in June and July.

By contraction and spreading, larva tears the chorion and then gently slides off the egg shell.

If not disturbed after hatching, larvae rest in the same place close to the chorion, which is of transparent white color and is still shaped like an egg.

Immediately after hatching, larvae are almost colorless to light beige, with denticulate body. Later, the color turns to brown.

During their growth, larvae shed two times and pass through three stages.

In the first larval stage (L1), the average weight is 1.49 mg (minimum recorded weight 0.4 mg/ maximum 2.1 mg), the average length 3.2 mm (min. 0.9 mm / max. 4.5 mm) and the average width 0.69 mm (Photo 1).

Photo 1. Larva after hatching

As the larvae grows, they become larger and in the second larval stage (L2) they turn brown, with expressed thorns on the segments.

In the second larval stage (L2), the average recorded weight is 5.1 mg (4.5 to 5.9 mg), the average length 5 mm (4 to 5.7 mm) and the average width 1.2 mm.

Toward its mouthpart the larva is pointed, dorsally slightly curved and ventrally flattened, with a pair of expressed, short brown stigmatic tubes.

In third larval stage (L3), larvae are yellow-brown or dark brown, with relatively long thorns along their body. Longitudinal stripe extends through the center of the dorsal side of the body. Larval respiration can be clearly observed through the cuticle. They have strong mouth hooks, suitable for catching the prey, sharp mouthparts like a dagger, strong pharynx and head muscles which help them to stab and suck the prey.

The average weight of larvae in L3 is 9.4 mg (ranging from 6.5 mg to 12 mg) and the average length is 7.01 mm (from 6 to 7.63 mm).

According to Ma et al. (1986), larval body is 6-7 mm long.

The width of larvae in L3 varies between 1.9 and 2.4 mm.

Larvae kill a great number of aphids. Their greed increases during the second, and particularly during the third larval stage.

When it finds an aphid, the larva stabs it from the outside or the inside and starts to feed on it. The aphid still moves its legs reflexively, while the larva with its mouthpart is penetrating its body, sucking all its content. Finally, the larva throws the wrinkled and dark aphid away.

The larvae move very fast, always touching the substrate with their heads and excreting secretes. With their sputum they moist the surface on which they crawl and fasten to tobacco plants.

When hungry, the larva sucks the first aphids completely and as it becomes satiated, it doesn't eat it thoroughly, but goes to find another aphid.

In our investigations the duration of larval stage is 8 days. According to Ma et al (1986) duration of larval stage is 7 to 8 days and according to Gao (1991) it is 10 days.

In 2003 and 2005, *P. guadrifasciatus* larvae were recorded in our tobacco fields in August and early September and the maximum was reached in August 20. In 2004, we observed single larvae from the second decade of July to the first decade of September.

According to Gao (1991), larvae can appear among aphids in the thick grass from late May to early September.

Larvae do not leave excrements frequently, but only before pupation. The excrements are black and they signalize that some larva in the laboratory or in field was transformed into pupa.

Larvae of *P. guadrifasciatus* are transformed into pupae in the same plants on which they feed: in the upside of tobacco leaves, in leaf sleeve or hidden among flowers and seed capsules.

In Petri dishes, larvae seek for suitable place for pupation in reverse side of leaves

or flowers, in hidden places far from light.

Puparium is formed from the last larval skin and its color and pattern resemble the 3rd stage. Immediately after pupation, the pupa is soft and its inside is still pulsating. Gradually, the skin of the pupae become firmer. They are yellow-brown and thorny. The rear part is slightly curved inwards and they use it to fasten to the substrate. The front part of the pupae is rounded and adults eclode from there.

The average pupal size is 5.04 mm (4.8 - 5.6 mm) in length and 1.93 mm (1.7 - 2.2 mm) in width, and the average weight is 8.93 mg (6 to 11.7 mg).

According to Ma et al. (1986), pupae are dark yellow, about 5 mm long (Photo 2).

Photo 2. Pupa

In our investigations, duration of pupal stage was 5-7 days. Gao (1991) reported a duration of 7-8 days, and Ma et al (1986) 12 days.

In tobacco fields, pupae were most frequently recorded in August and early September.

Before the eclosion of imago, the pupa becomes darker. In our investigations it could be stated that eclosion takes place early in the morning. As the head of imago presses the puparium, it cracks in a form of circle and its upper part opens like a lid. The imago comes out of the pupa and stands still.

Immediately after eclosion, the cuticle of the imago is very soft and delicate, but gradually it becomes firmer. In the beginning, wings are shaped like small triangles but gradually they open, stretch and dry. The wings are delicate and soft and their nervature is clearly recognizable. The patterns and color of the body develop in few hours. After eclosion, the abdomen is empty, but it gradually obtains its form.

This species is among the smallest syrphid flies. The imagos are small, considerably short and dark. The head is round and wider than the thorax. The complex eye is dark brown. As in other hoverflies, sexual dimorphism is present, but the gender can be easily differentiated. The eyes in females are separated and in males they are merged or very close together.

The antenna is brown and its III segment is 2.5 times longer than the sum of the first and second segment. The mesotergum is dark blue, with metallic shine. There is a pair of short longitudinal stripes in the first half and yellow-brown hairs on both sides. Scutelum is black in the first half and yellow in the second. The foot is yellow-brown.

The abdomen is black and its first and second segment are also black. The other abdominal segments have visible yellow-white lateral stripes. The stripes on the last two segments are narrower and interrupted in the middle.

Typical for this species is that its body is somewhat curved in the lower part, i.e. the abdomen is curved downward.

The average length of females is 5.43 mm, varying from 4.5 to 6.7 mm and the width varies from 2 mm to 2.5 mm (Photo 3).

Males are somewhat smaller than females, with a length of 4.5 mm and width 1.7 - 2 mm (Photo 4).

Photo 3. Female of *P. quadrifasciatus*

Photo 4. Male of *P. quadrifasciatus*

According to Bankowska (1963), the imago of *P. guadrifasciatus* is 6 mm long, while according to Ma et al. (1986) its length is 5 - 6 mm.

In our laboratory investigations of the imagos of *P. guadrifasciatus* some deviations were recorded in abdomen color and the abdominal pattern of the adults was incomplete. This was also observed in our field investigations. It was confirmed, however, that it was the same species.

In laboratory conditions, the imago lives 6-7 days.

Sexual index in the investigated years (2003-2005) was 0.54, which indicates that females are somewhat more numerous than males.

In our investigations, the growth of one generation from egg to imago was 16-19 days, Ma et all. (1986) reported a period of 17 - 20 days and Gao (1991) 25-30 days.

CONCLUSION

P. guadrifasciatus is among the smallest syrphid flies. Its larvae are known predators of leaf aphids.

The imagos are small, considerably short and dark. The abdomen is black and its first and second segment are also black. The other segments of the abdomen have two visible yellow-white lateral stripes. The stripes in the last two segments are narrower and interrupted in the middle. Typical for this species is that its body is somewhat curved in the lower part.

The eggs are oval and cream-colored. Duration of egg stage is approximately 3 days.

Immediately after hatching the larva is almost colorless to light beige, with toothed surface. As the larva grows, it becomes brown, with expressed thorns on the segments. The larval stage usually lasts 8 days.

Pupae are yellowish-brown and prickly and the duration of pupal stage is about 5-7 days.

In laboratory conditions imago stage lasts 6 -7 days.

Sexual index in the investigated years (2003-2005) was 0.54, which indicates that females are somewhat more numerous than males.

According to our investigations, the growth of one generation from egg to imago was 16-19 days.

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INVESTIGATION INTO THE TYPES OF PIPE TOBACCO

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ABSTRACT

The European commission community highlights the importance of development for the standards of tobacco products control including the cigarettes. Pipe tobacco has not been under any control and surveillance yet. Thus, there is a lack of information, methods and apparatus for observing the emissions of harmful substances in smoke. There is also a lack of established conditions, rules and any control.

The purpose of this research is to discover the importance and essence of modern types of pipe tobacco, the level of preference and possibilities for their control.

The first preliminary studies of pipe tobacco provide direction for further research as there is a high possibility for increasing their consumption.

Key words: tobacco, tobacco products, control.

ИСПИТУВАЊЕ НА НЕКОИ МАРКИ НА ТУТУН ЗА ЛУЛЕ

Во извештаите на Комисијата на Европската заедница се истакнува важноста за развивање на стандарди за контрола на тутунските производи, вклучувајќи ги и цигарите.

Тутунот за луле досега не бил подложен на никаков надзор и контрола. Поради овие причини, не постојат какви било информации, методи и апаратура за следење на емисиите на штетните елементи од чадот. Исто така, не постојат утврдени услови и недостасува регулатива и контрола.

Целта на ова истражување е да се открие важноста и суштината на модерните типови на тутун за луле, нивото на одредени параметри и проучување на можностите за нивна контрола.

Овие први, прелиминарни студии на тутунот за луле даваат насоки за подлабоко истражување, затоа што постои голема веројатност за зголемување на нивната консумација.

Клучни зборови: тутун, тутунски производи, контрола

INTRODUCTION

As a member of the European Community, Bulgaria has to fulfill a number of tasks assigned by the European Parliament, Commission and Council regarding the forthcoming changes in Directive 2001/37/EO. In the reports of the Commission of the European Community as well as before the European Parliament, World, European Economic and Social Committee have been again emphasized the importance of targeted research on developing standards for tobacco products control including cigarettes (1,2,3).

Pipe tobacco has not been under any control and surveillance so far. Moreover, until recently, it was thought that the form of tobacco product was outdated not meeting the dynamism of our times. Thus, there is a lack of information, methods and apparatus for observing the emissions of harmful substances in smoke. There is also a lack of established conditions, rules and any control.

In recent years, the tobacco for hand rolling cigarettes or rather RYO tobacco has been an alternative to the industrial production. At the same time, the outspread types of cut pipe tobacco have recorded growth in consumption. *According to this fact and its characteristic of full flavored taste lead to us to think that the type of pipe tobacco may be more preferred than hand rolling cigarettes.*

Purpose

The aim of this research is to discover the essence and importance of modern brands of tobacco for pipes, the degree of preference and the opportunities for their control.

MATERIAL AND METHODS

The focus of our studies was on the preferred brand of tobacco pipe on the Bulgarian market. In order to achieve the goal of the task, database was created by conducting monitoring for the preferred consuming brands in 2009. The subject of our further research was the subsequent data processing and the specification of brands.

An expert and tasting assessment was made on the contingent of tobacco pipe. This was followed by full physical-chemical analysis of samples, processing of results and interpretation based on standardized methods.

RESULTS AND DISCUSSION

The results of the conducted monitoring on the orientation of tobacco products consumers are visualized in figure 1.

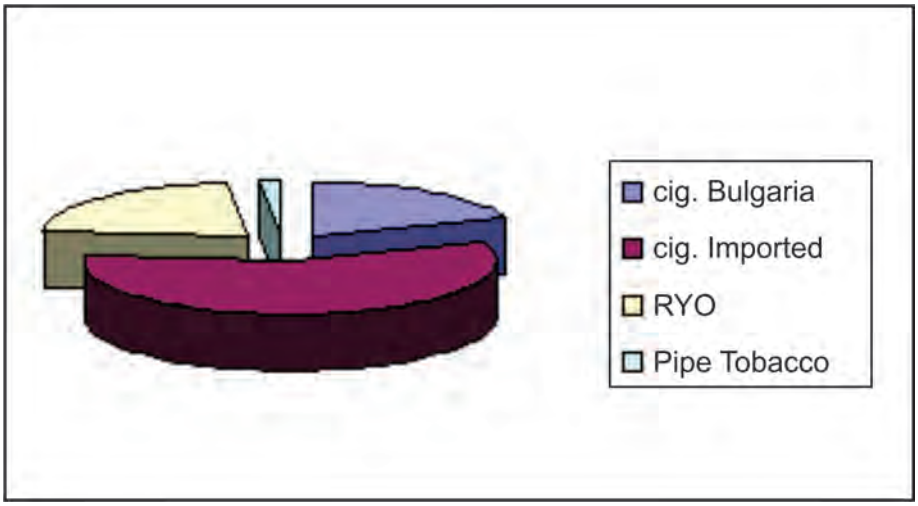

Fig.1 Consumer preferences of tobacco products

When comparing the data with 2008, there is an increasing demand for pipe tobacco illustrated in Table 1 and Figure 2.

Table 1 Percentage of preferred tobacco products in 2008 and 2009

Tobacco products	2008	2009
Cigarettes – Bulgarian Industry	19,97	19,21
Imported cigarettes	57,81	57,49
RYO	21,60	21,67
Pipe tobacco	0,62	1,63

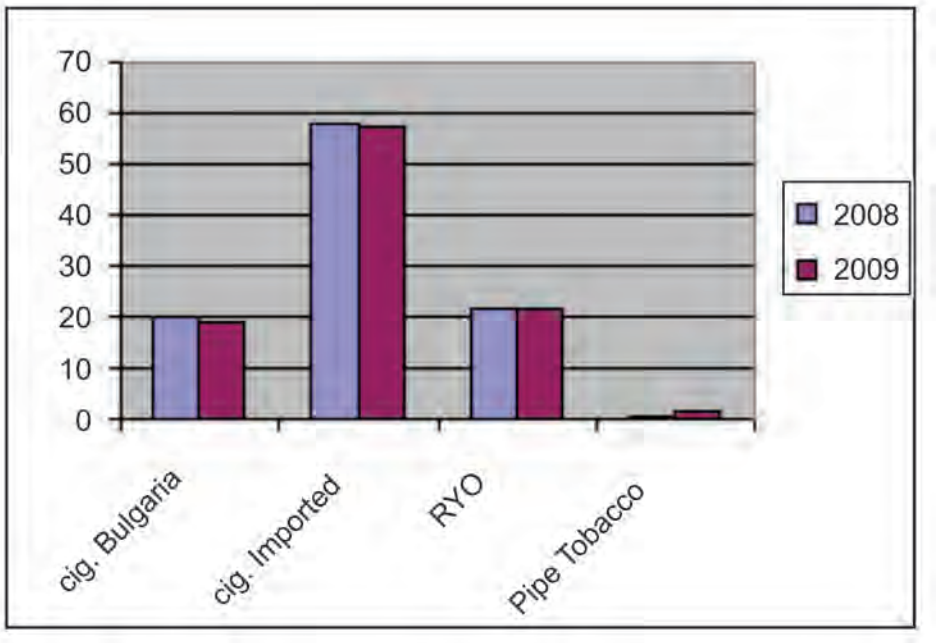

Fig.2 Percentage of preferred tobacco production in 2008 and 2009

The increasing of pipe tobacco consumption is very important for the reduction in expense of Bulgarian cigarettes – 0.76% whereas imported cigarettes are half reduced compared to the Bulgarian – 0, 32%. It is a noteworthy fact that the share of tobacco for rolling cigarettes and the share of consumed pipe tobacco rise at approximately same percentage. The growing interest in pipe tobacco can be primarily attributed to the full and filling flavor. It is economically more favorable than the hand rolling tobacco of cigarettes and it is almost equal to, with a slight more economic benefit in some brands of industrially manufactured cigarettes.

The consumers of pipe tobacco can be divided into two groups. The first are those according to the above-mentioned reason. The latter are those fond of pipes as an alternative to the hectic pace of life. The lighting of a pipe is a ritual signifying time for rest and reflection. There are also some who want to display stability by doing it. There have lately been found even associations in the logo of the pipe.

The overall conclusion of the monitoring is that the increasing consumption of pipe tobacco is not due to the ever growing alternative of hand rolling cigarettes.

It should be emphasized that the results of the conducted monitoring cannot be taken as statistically significant. The essence of these results lies in establishing a perceived tendency in change of tobacco products consumption in recent years. Even as a proof, it can be taken the Bulgarian market which is no different than the world market in terms of dynamics and directions.

While conducting the monitoring, many brands of pipe tobacco were also taken into consideration. This fact shows that there is no categorical view, or rather existing tradition as the selection is based on poll questions.

The general conclusion of the examination of the required brands for pipe tobacco is that the brands produced in Germany, Denmark and the Netherlands will be at an advantage compared to other brands. The results of the use of preferred brands of pipe tobacco are graphically shown in Figure 3.

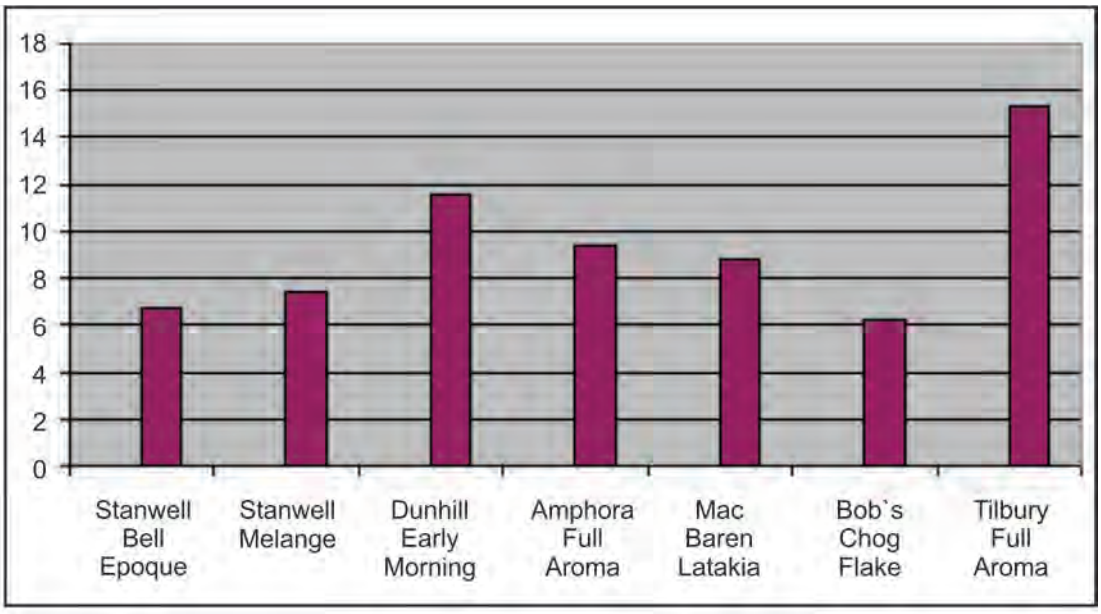

Fig.3 Preferred pipe tobacco brands

While processing the results, 38 brands of pipe tobacco were observed in 2009, but graphically are shown only those whose consumption is greater than 6%. From the image, it can be noted that the most dominant brand is Tiliburu Full Aroma of Poshl Tabak, Germany at 15, 33% which determined the object of further research. The results of the made expert assessment of Tiliburu Full Aroma are as follows;

The Packaging consists of 40 grams of excise band and a recommendation for pipe tobacco priced at 8, 00 lev. On the front side, there are warning and special labels, producer and importer, brand name, prepared for realization, bar code. Unlike the tobacco for hand rolling cigarettes, packed units meet all required standards.

The tobacco has quite low values, with width of 1,77 mm incision and vary from 1,36 mm to 2,18 mm, with moisture content of 16,61 %. The tobacco contains a small percentage of processed tobacco ribs at 7, 42%.

When opening the packing, the aroma is intense at a higher average level, fresh, quite dark, with a character of cherry and rum-like – richly hued. While smoking, the cherry-like hue is dominant, and the taste is full at a medium level. A general conclusion can be drawn that it is tobacco with a balanced tasty and aromatic complex.

The results of the chemical composition of tobacco with its basic parameters are as follows:

Nicotine ……..2, 31%
Reducing sugars ……...10,10 %
Total nitrogen ……2, 51%
Content of ashes …….. 14, 76%

The results of the basic chemical parameters in the studied tobacco are close to the values found in tobacco for hand rolling cigarettes.

In order to explore the possibilities for pipe tobacco control, the further research was based on laboratory-made cigarettes compared with pipe smoking. The smoking cigarettes were made without filter and with a precise weight. The first variant of cigarettes were made of poorly combustible and impermeable paper with a mass of 0,500 g. The second variant of cigarettes was made of industrial paper for cigarettes with air permeability of 50 CU and flammability of 55 s. The third variant of cigarettes was made of cigarettes' spill Orient Ehpres i.e. an imitative cigarillo. The last two variants were with a mass of 1, 00 g. Because of a lack of a method, we emphasized on creating burned tobacco in various conditions. According to this, in the first variant, we created conditions which are close to those of pipe smoking. In the second variant, the conditions are close to those of controlled products (cigarettes) and the conditions in the third variant are close to the conditions of cigarillos and cigars based on ISO standard.

The purpose of this variant was to give us insight into any future research and discovering possibilities for controlling the consumed smoke while pipe smoking. The pipe itself helped us for the control. Of the three studied elements in smoke such as tar, nicotine and CO, we stressed mainly the content of tar in smoke. The top of one standard pipe can be filled with tobacco of 4 grams. In order to make a comparison during the research, the pipe was filled with tobacco of 1 gram in eleven iterations. The average arithmetic value of tar content was 0, 03600 grams.

In the first variant, the estimated amount of tar per unit of burned tobacco was 0, 03658 grams. In the second variant, the estimated amount was 0, 01654 grams and in the third variant 0, 04352 grams. During the control of the first variant, it was recorded a slight increase in the amount of tar. On the one hand, this is mainly because of the impact of the paper which takes part in the combustion and it is incombustible and impermeable. On the other hand, the smoke goes throughout the stem of pipe and it is very likely that an amount of it remains on its walls.

In the second variant, the amount of tar was significantly with smaller value. In this case, it was used a paper with medium permeability which had a considerable influence upon the content of tar. In the third variant, the amount of tar significantly exceeded the control. This is due to the specificity of the cigarette paper which was impredned with tobacco extract**.**

The results of the third variant are of significant importance for this research as they showed a tendency for further analysis and give us an opportunity to invent new methods for pipe tobacco along with the existing ones for cigars and cigarillos.

CONCLUSIONS

This initial study on pipe tobacco gives us directions for further research. From the obtained results, it is reasonably safe to assume that the pipe tobacco cannot substitute the tobacco for hand rolling cigarettes. Its control is compulsory and possible as well. This research serves as a direction for carrying out future ones.

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BIOLOGICAL EFFECT OF SOME SOIL HERBICIDES IN VIRGINIA TOBACCO

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ABSTRACT

During 2008-2009, a randomized field experiment was conducted with 4 soil herbicides on an alluvial-meadow soil in the village Karadzhalovo, Bulgaria. The experiment was conducted with flue cured tobacco variety Virginia 0454, on a soil which had been previously planted with wheat. It consisted of Devrinol 4 F at a dose 4,0 l/ha (a.s. napropamide); Pronin 720 EC at a dose 2,5 l/ha (a.s. propizohlor); Stomp New 33 EC at a dose 4,0 l/ha (a.s. pendimethalin); Harness at doses 1,5 l/ha and 2,5 l/ha (a.s. acetochlor).

In the areas with herbicides, one mechanized processing in line spacings and one hand hoeing in the rows were carried out. In the control without herbicides, two mechanized and two manual handlings of the soil were made. The preparations were applied 24 hours before the tobacco planting without incorporation.

Stomp New 33 EC in a dose 4,0 l/ha and Pronin 720 EC in a dose 2,5 l/ha showed very good to excellent efficacy against susceptible annual weeds. Pendimetalin and propizohlor help to increase the yield from 39,58% to 40,47% and to increase the average percentage of Ist class Virginia tobacco for 6,5%. When conditions for growth and development of flue cured tobacco are more favorable, the application of herbicides has less effect on yield increase. The application of soil herbicides before tobacco planting allows a reduction of manual and mechanical hoes, without an adverse effect on the culture.

Key words: herbicides, Devrinol 4F, Stomp 33 EC, Harness, Pronin 720 EC, virginia tobacco, weeds

БИОЛОШКИОТ ЕФЕКТ НА НЕКОИ ПОЧВЕНИ ХЕРБИЦИДИ ВРЗ ТУТУНОТ ОД ТИПОТ ВИРЏИНИЈА

Во селото Карачалово, Бугарија (2008-2009) извршен е рандомизиран полски експеримент со 4 почвени хербициди на алувијално-ливадска почва со тутун од сортата вирџинија 0454 (на површина на која претходно е одгледувана пченица): Devrinol 4F со доза од 4,0 l/ha (а.м. napropamide); Pronin 720 EC со доза 2,5 l/ha (а.м. propizohlor); Stomp (New) 33 EC со доза од 4,0 l/ha (а.м. pendimethalin); Harness со доза од 1,5 l/ha и 2,5 l/ha (a.m. acetochlor). На третираните површини беше извршено едно механизирано меѓуредово окопување и едно рачно окопување во редовите. Во контролата без хербициди беа извршени 2 механизирани и 2 рачни окопувања на почвата. Препаратите беа аплицирани 24 часа пред расадувањето на тутунот, без инкорпорација.

Stomp (New) 33 EC со доза од 4,0 l/ha и Pronin 720 EC со доза од 2,5 l/ha имат добра до одлична ефикасност против едногодишните плевели. Пендиметалинот и пропизохлорот овозможија зголемување на приносот од 39,58% до 40,47 %, како и зголемување на процентот на 1-та класа на тутунот во просек од 6,5 %. Кога условите за раст и развиток на вирџинијата

се поповолни, примената на хербицидите има помал ефект врз зголемувањето на приносот. Примената на почвените хербициди пред садењето на тутунот овозможува намалување на рачните и механизираните окопувања, без какви било негативни ефекти на културата.

Клучни зборови: хербициди, Devrinol 4F, Stomp 33 EC, Harness, Pronin 720 EC, тутун од типот вирџиниј, плевели

INTRODUCTION

The tobacco production in Bulgaria includes many manual operations. This makes this sector of Bulgarian agriculture insufficiently profitable and competitive. One of the mandatory elements of the new, more efficient and modern technologies is the streamlining of the systems for pest management in tobacco against the weeds.

According to FAO, the losses caused by weeds in tobacco in the world amounted to 37% of total production. The results of experiments in Bulgaria, conducted in different ecological conditions indicate that this percentage is even higher and reached 42 points. When the weed density is even 2 - 3 numbers weeds/m^2 during the growing season of the crop, the yield fells from 14% to 18%. The harmful effect of the weeds in tobacco is not only a reduction of the yield. The weed infestation affects the quality of production and makes difficult or impossible the mechanization of the production and supporting the development of diseases and pests.

The chemical control against the weeds in comparison with the mechanical destruction methods has significant advantages. The chemical method is more effective compared to mechanical processing of the rows and hand hoeing. The herbicides allow the application of a rational technology for growing of the crops. That is one of the main factors for determination of the quality of tobacco leaves / 2, 5, 6 /.

Therefore, the continuous streamlining of the systems for weed control in tobacco is not only of scientific but also of important practical significance.

Furthermore, the use of environmentally friendly systems of herbicides is an integral part of the good plant protection practice in any culture. Solving the problem of weeds in tobacco requires regular review of new herbicides, of their biological efficacy and their influence on the yield and the quality of the culture.

The aim of the study was to establish the biological effectiveness and the spectrum of action on 4 soil herbicides in Virginia tobacco, to study the influence of herbicides on tobacco yield and the quality and to explore opportunities to reduce the vegetation treatments of soil by the use of herbicides.

MATERIAL AND METHODS

The field experiments were conducted during 2008 – 2009 year on alluvial-meadow soil in the village Karadzalovo, Bulgaria in flue-cured tobacco variety Virginia 0454, after wheat as a precrop. The humus content in the test area at depths of 0 to 30 cm averaged 2,5 %. The field moisture of the soil at the time of treatment was 34-36%.

The random field experiment included six options in four replications. The size of the test area was 100 m^2 (Barov, Shanin, 1965). Four soil herbicides were investigated: Devrinol 4 F in a dose 4, 0 l / ha (a.s. napropamide); Pronin 720 EC in a dose 2,5 l / ha (a.s. propizohlor); Stomp New 33 EC in a dose 4,0 l / ha (a.s. pendimethalin); Harness at doses of 1,5 l / ha and 2,5 l / ha (a.s. acetochlor). The control was without herbicides. One mechanized processing in the line spacing and one hand hoeing in the rows have being made in the areas with herbicides. Two mechanized and two manual handlings of the soil were carried out in the control areas. The herbicides were brought 24 hours before the tobacco planting without incorporation. The treatment was carried out

with a knapsack sprayer at expense of working solution 300 l / ha.

The yield was statistically processed by analysis of variance (Zapryanov, Marinkov, 1978). The proof of the differences between the versions is established by Dunkan test (1995, cit. Johnson, 2008) at a significance level α=0,05 (5%) and 0,01(1%). To establish the biological efficacy of the investigated herbicides the following indicators were reported: Species composition of weeds - the reading on 28^{-th} and 45^{-th} day following the preparations at fixed sites 1 m^2, in four repetitions. Density of weeds - the reading is a quantitative method / number of weeds on 1 m^2 / on 28^{-th} and 45^{-th} day following the preparations at fixed sites 1 m^2, in four repetitions. To establish the impact of herbicides on tobacco, the following parameters were reported: Yield - kg/dka dry tobacco; Quality - I, II, III class in percentages.

RESULTS AND DISCUSSION

Stomp New 33 EC in a dose of 4,0 l/ ha shows excellent efficacy in both years of the study. In 2008, 28 days after the introduction of the preparation, 96% to 98% sensitive weeds were killed (Table 1) and in 2009 – 94,5% to 97,5% of them (Table 2). At field conditions, Stomp New 33 EC demonstrated good duration of herbicide action. 45 days after the treatment, efficacy of the preparation was with score 1-2 by EWRS in both experimental years. At the same time, in plots without herbicides and with two hoes, weed infestation was 31,5 – 33,5% higher (Table 1 and 2). These data confirmed the results of other experiments that pendimetalin has excellent efficacy against Digitaria sanquinalis Scop., Echinohloa crus-galli L., Setaria viridis L.(P.B.) and Setaria glauca P.B. and very good efficacy against Amaranthus retroflexus L. and Amaranthus blitoides L., good efficacy against Portulaca oleracea L. and Chenopodium album L. Abutilon theophrasti Medic., Xantium srumariun L. and Datura stramonium L. are resistant.

Pronin 720 EC in a dose of 2,5 l/ha shows very good efficacy against annual wheat weeds and very good efficacy against annual broad-leaf weeds. In 2008, 28 days after its submission, 95,5% to 97,5% of the weeds were killed. (Table 1) and in 2009 94% to 96% of them (Table 2). 45 days after the treatment, efficacy of the preparation was with score 1-3 by EWRS in experimental years. At the same time, in the plots without herbicides and with two hoes, weed infestation was 28,5%–31,0% higher (Table 1 and 2).

Propizohlor, as demonstrated by other studies, has excellent efficacy against the annual wheat weeds - Setaria ssp., D. sanquinalis, E. crus-galli, and very good efficacy against A. retroflexus and A. blitoides. The effect is weaker against P. oleraceae and C. album.

All herbicides were sprayed only 24 hours before planting without an incorporation, but they had no inhibitory effect on the growth and development of tobacco variety Virginia 0454.

Moreover, the application of Stomp New 33 and Pronin 720 EC allows the reduction of the manual and the mechanical hoeing without adverse effect on the weed infestation and on the culture.

It should be mentioned that Harness in a dose 2,5 l/ha caused some inhibition of growth and development of the culture, which has been overcome till the end of the vegetation period of tobacco.

Table 1. Biological efficacy of herbicide preparations in 2008

Herbicide preparations	Dose, l/ha	% of destroyed weeds						EWRS efficacy
		28 days after treatment			45 days after treatment			
		wheat weeds	broad-leaf weeds	average% of destroyed	wheat weeds	broad-leaf weeds	average% of destroyed	
Pronin 720 EC	2,5	97,5	95,5	96,5	95,0	93,0	94,0	1-2
Devrinol 4 F	4,0	91,5	94,5	93,0	90,0	94,0	92,0	1-3
Stomp New330 EC	4,0	98,0	96,0	97,0	97,0	96,0	96,5	1-2
Harness	1,5	94,0	91,0	92,5	88,0	86,0	87,0	2-4
Harness	2,5	97,5	93,0	95, 5	91,0	85,0	88,0	1-4
Control	-	93,0	93,0	93,0	60,0	66,0	63,0	-

Table 2. Biological efficacy of herbicide preparations in 2009

Herbicide preparations	Dose, l/ha	% destroyed weeds						EWRS efficacy
		28 days after treatment			45 days after treatment			
		wheat weeds	broad-leaf weeds	average% of destroyed	wheat weeds	broad-leaf weeds	average% of destroyed	
Pronin 720 EC	2,5	96,0	94,0	95,0	94,0	90,0	92,0	2-3
Devrinol 4 F	4,0	90,0	92,0	91,0	89,0	92,0	90,5	2-4
Stomp New330 EC	4,0	97,5	94,5	96,0	95,0	95,0	95,0	1-2
Harness	1,5	92,0	92,0	92,0	89,0	89,0	89,0	2-4
Harness	2,5	94,0	94,0	94,0	92,0	91,0	90,5	2-4
Control	-	92,0	92,0	92,0	65,0	62,0	63,5	-

The results for the influence of the soil herbicides on yield and the quality of tobacco are presented in Table 3 and Table 4.

In 2008, the highest yield was obtained in options Stomp New 330 EC in a dose 4,0 l /ha. It was 269,7 kg/dka, which is 40,47% higher yield compared to the control. Pronin 720 EC increases the yield for 39,58%, compared to the control.

In 2009 the results were similar. The highest yield had Stomp New 330 EC - 333,8 kg/dka, followed by Pronin 720 EC - 293,0 kg/dka. It is noteworthy that in 2009 the relative increase of the yield was less. In the new version of Stomp 330 EC and Pronin 720 EC it is respectively 29,37% and 16,73%, compared to the control.

The analysis of these data shows that in the year with more favorable conditions for growth and development of tobacco, as well as in 2009, the positive effect of the herbicides on the yield of tobacco is less pronounced. For example, in 2008 the increase of the yield ranged from 0,78% to 40,47% and in 2009 - from 1,19% to 29,37%. The highest rate of tobacco Ist class in both years was obtained with variants Pronin 720 EC – 59,0% in 2008 and 55,5% in 2009, followed by Stomp New 33 EC - 57,5% in 2008 and 54,0% in 2009. The percentage of first class tobacco is higher in all variants treated with herbicides, compared to the untreated control.

Table 3 Yield and quality of tobacco variety Virginia 0454 in 2008

Herbicides preparations	Dose, l/ha	Yield of tobacco			quality of tobacco, %		
		kg/dka	distinction to the control	Relative yield, %	I class	II class	III class
Pronin 720 EC	2,5	268,0	76,0	139,58	59,0	29,0	12,0
Devrinol 4 F	4,0	266,7	74,7	138,90	55,0	32,0	13,0
Stomp New330 EC	4,0	269,7	77,7	140,47	57,5	34,5	8,0
Harness	1,5	261,9	69,9	136,40	54,0	30,5	15,5
Harness	2,5	193,5	1,5	100,78	40,0	25,0	35,0
Control		192,0		100	49,0	25,0	26,0

Table 4 Yield and quality of tobacco variety Virginia 0454 in 2009

Herbicides preparations	Dose, l/ha	Yield of tobacco			quality of tobacco, %		
		kg/dka	distinction to the control	Relative yield, %	I class	II class	III class
Pronin 720 EC	2,5	293,0	42,0	116,73	55,5	31,0	13,5
Devrinol 4 F	4,0	288,4	37,4	114,90	54,0	32,0	14,0
Stomp New330 EC	4,0	333,8	82,8	129,37	54,0	34,5	11,5
Harness	1,5	272,8	21,8	108,68	54,0	32,0	14,0
Harness	2,5	254,0	3,0	101,19	41,0	30,0	29,0
Control		251,0		100	49,5	30,0	20,5

CONCLUSIONS

1. Stomp 33 New EC (a.s. pendimetalin) in a dose of 4,0 l ha/ and Pronin 720 EC (a.s. propizohlor) in a dose of 2,5 l / ha, applied 24 hours before planting of the flue cured tobacco showed good to excellent efficacy against susceptible annual weeds. The efficacy of the preparations was with a score from 1 to 3 by EWRS in both experimental years.
2. Pendimetalin has excellent efficacy against D. sanquinalis, E. crus-galli, S. viridis and S. glauca. Pendimetalin has very good efficacy against A. retroflexus and A. blitoides and good efficacy against P. oleracea and C. album. A. theophrasti, X. srumariun and D. stramonium are resistant.
3. Propizohlor has excellent efficacy against annual wheat weeds - Setaria ssp., D. sanquinalis, E. crus-galli and very good efficacy against A. retroflexus and A. blitoides. The effect is weaker against P. oleraceae and C. album L.
4. Pendimetalin and propizohlor help to increase the yield average for 27% and to increase the percentage of Ist class of Virginia tobacco average for 6,5%.
5. When conditions for growth and development of tobacco are more favorable, the application of herbicides has less effect on increasing of the yield.
6. The application of soil herbicides before planting of tobacco allows a reduction of manual and mechanical hoes, without adverse effect on weed infestation and on the culture.

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EFFECTS OF THE HERBICIDE NAPROPAMIDE ON THE SOIL MICROFLORA IN TOBACCO BEDS

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ABSTRACT

Using herbicides has a significant effect on different microbial processes in the soil and, depending on the type of soil and on the used dose, they may modify the quantity of their biomass for a certain period of time.

The aim of this investigation was to trace and identify the effects of the herbicide napropamide on the principal groups of microorganisms in the soil during the production of seedlings for broad-leaf tobacco, as well as the impact of weather conditions on this process.

Soil samples were taken on the 1-st, 7-th, 15-th, 30-th and 60-th days after input of the product. It was found out that Devrinol 4F has a direct influence on major groups of soil microorganisms. Thirty days after treatment this effect is insignificant.

Key words: herbicides, soil, tobacco, Devrinol 4F

ВЛИЈАНИЕТО НА ХЕРБИЦИДОТ НАПРОПАМИД ВРЗ ПОЧВЕНАТА МИКРОФЛОРА ВО ТУТУНСКИТЕ ЛЕИ

Употребата на хербицидите има значително влијание врз различните микробиолошки процеси во почвата. Во зависност од типот на почвата и применетата концентрација, тие можат да ја изменат количината на микрофлората за одреден временски период.

Целта на ова истражување беше да се утврди влијанието од хербицидот напропамид врз поважните групи на микроорганизми во почвата за време на производството на расад од крупнолисни тутуни, како и влијанието на временските услови врз овој процес.

Примероци од почвата се земани на 1-, 7-, 15-, 30- и 60-от ден по внесувањето на препаратот.

Констатирано е дека Девринол 4Ф има директно влијание врз главните групи на почвени микроорганизми. По 30 дена од третирањето со препаратот, влијанието на хербицидот е незначително.

Клучни зборови: хербициди, почва, тутун, Девринол 4Ф

INTRODUCTION

Tobacco is a traditional crop of social importance that has been grown in our country for centuries. Considering climate conditions and relief, there are several tobacco growing regions in Bulgaria under different types and varieties of tobacco.

The production of tobacco depends on the production of high quality and healthy seedlings. The application of herbicides is a compulsory measure in the production of tobacco seedlings. The high rate of weeds in seedling beds is determined by various factors such as; use of manure, limited use of herbicides, limited planting areas, and so on. Napropamide is one of the selective herbicides approved for use in tobacco seedlings (marketed as Devrinol 4F). As a selective systematic herbicide, napropamide is efficient against a number of annual wheat weeds and some dycotyledons. The herbicide is absorbed by the roots and works by inhibiting root development and growth.

Napropamide is resistant to drought. In most soils of mineral composition, it is degraded by microorganisms at a much slower rate (8). Furthermore, it has been determined that half of the initial input dose in soils of light mechanical composition degrades under the influence of biotic and abiotic factors between day 9 and day 17. In higher doses, the herbicide's activity remains for more than six months. In some cases, it leads to initial slowing down in the growth of the next crop in the crop rotation.

Research in our country and abroad has proved that herbicides have an impact on different microbial processes in the soil and, depending on the type of soil and on the used dose, they may modify the quantity of their biomass for a certain period of time. It has been established that the use of pesticides (herbicides) has a selective inhibition effect on the microorganisms which are responsible for nitrogen fixation and nitrification from 4 to 12 weeks. The use of herbicides reduces the total microbial population in the soil (5), whereas this can be due to reduced input of organic waste as a result of the fight against weeds. Pesticides are decomposed faster in soils with higher organic matter content, probably due to higher microbial activity.

The main microorganisms that take part in the decomposition of herbicides are bacteria, actinomycetes and microscopic fungi.

The purpose of this study was to trace and identify the effects of the herbicide napropamide on the major groups of microorganisms in the soil during the production of seedlings for broad-leaf tobacco, as well as the impact of weather conditions on this process.

MATERIAL AND METHODS

The field experiment was conducted in 2007 and 2008 on tobacco beds within the experimental field of the Tobacco and Tobacco Products Institute in the village of Markovo, the region of Plovdiv. The trial was planted according to the iambic standard scheme in 5 repetitions of 1m^2 experimental lots on humus-carbonated soil. The pH of the soil was 7.7, the humus content – 2.32% (Turin), total nitrogen – 0.182 mg, phosphorus - 2.68 mg/100 g and potassium - 42.79 mg/100 g.

The trial period included years with different average temperature and quantity of precipitations, unevenly distributed throughout the vegetation period. The periods of seedling production over the two years differed significantly by the quantity of precipitations (see Table 1).

Devrinol 4F was applied in the soil using portable sprayer in doses of 400 ml/da twenty-four hours before planting the tobacco seeds. Soil samples were taken for microbiologic analyses on day 1, day 7, day 15, day 30 and day 60 after the product application. The mass of each sample ranged between 600 and 800 g; the samples consisted of 5 to 7 extracts randomly taken from the soil at a depth between 0 and 15 cm.

Microorganisms were measured according to the dilution method and by planting on selective nutrient media. Their quantity is represented as column forming units. The total biological activity was studied as well by measuring the emissions of CO_2 (9). Determination was made on the relation between the number of microorganisms and the time of treatment.

Table 1 Precipitations in mm

Precipitations in mm	2007			2008		
	April	May	June	April	May	June
1-10	13.2	9.5	139.8	46.8	20.9	26.2
11-20	0.9	6.9	3.4	12.9	0	34.9
21-31	1.2	122.1	0	20.6	29.7	1.4
Σ mm^2	15.3	138.5	143.2	80.3	50.6	62.5

RESULTS AND DISCUSSION

Since herbicides are biocide substances, they have an impact on microorganisms manifested in increasing their productivity and adaptation capacity. Therefore, the dynamics of changes in soil microflora is the main parameter for evaluation of the importance of main environmental components in agro-biocenosis after the use of herbicides.

Amonifying bacteria are indicators of the activity of mineralization of the organic substances in the soil. They are distinguished among bacteria developing on mesopeptonic agar for being the most resistant to the composition of the soil. The data in Fig. 1 show the changes in population dynamics of the studied group under the influence of herbicide. Their quantity is inhibited on day 7 after input of Devrinol 4F, which is believed to be due to direct toxic effect on bacteria.

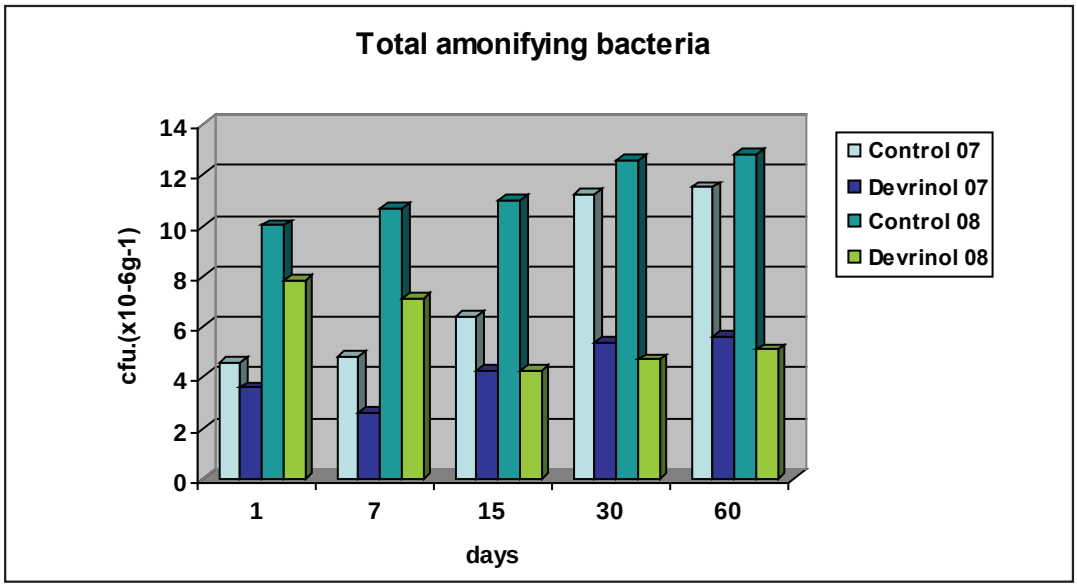

Fig. 1: Number of bacteria that degrade organic nitrogen compounds

This effect is gradually overcome and their number increases at every subsequent stage of the study. The trend remains the same over the two years of the experiment.

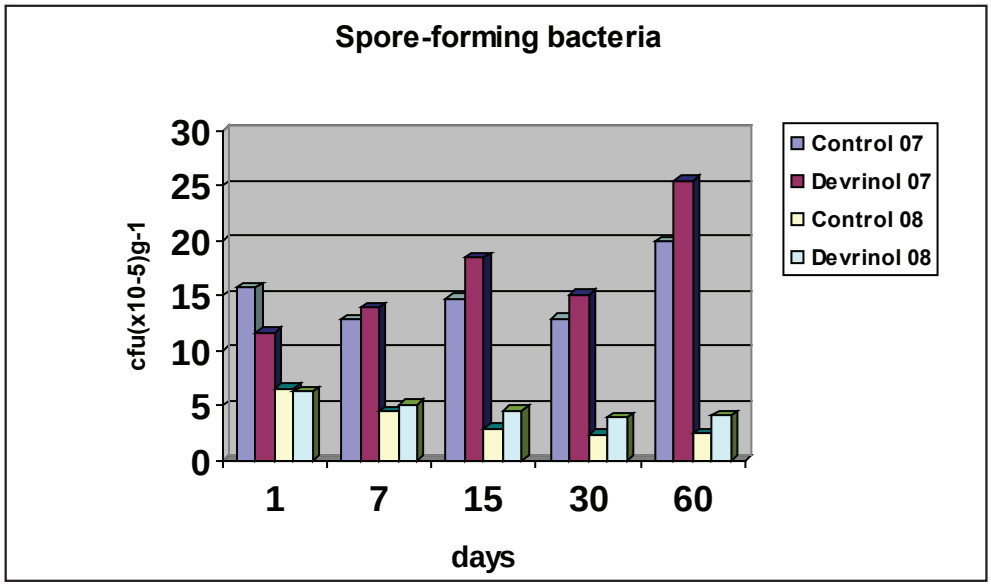

Fig. 2: Number of spore-forming bacteria

The results shown in Fig. 2 demonstrate the dynamics of variation of the number of spore-forming bacteria. Their quantitative expression results from the intensity of mineralization processes in the soil. The tendency of decreasing the number of spores up to day 7 proves that Devrinol 4F in doses of 400 ml/da has a positive effect on this group of bacteria. Over the following periods, it was established that spore formation is stimulated, which means that the decomposition of the herbicide was not complete at the time of the last sampling, i.e. 60 days after its input. The number of spores varied within a wide range in 2007 when spore formation reached higher values. On its part, this leads to decelerating the transformation of some organic compounds in the soil and slower mineralization processes.

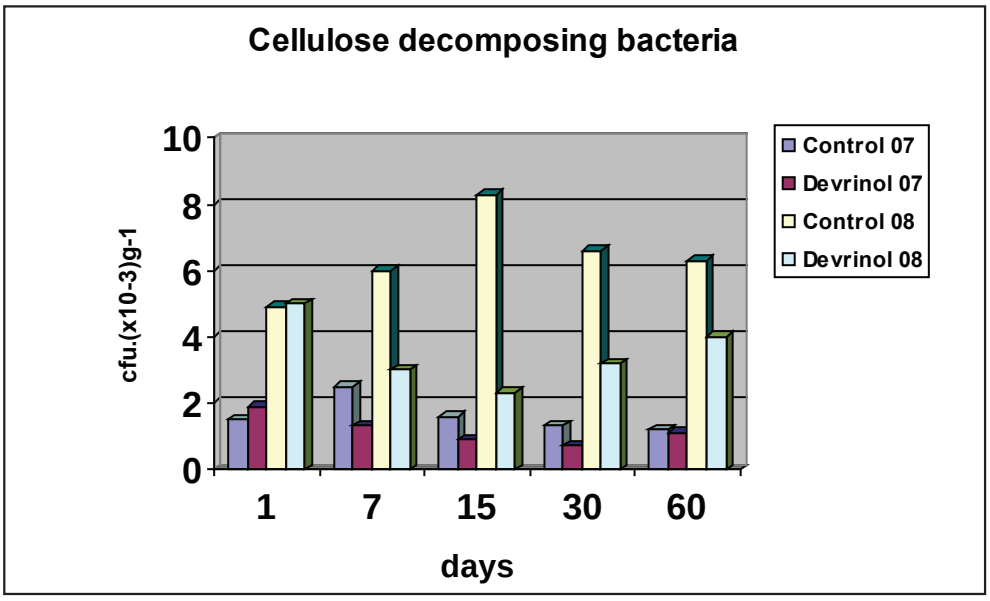

Fig. 3: Cellulose decomposing bacteria

In experimental conditions Devrinol 4F had a direct impact on the number of cellulose decomposing bacteria in the soil (see Fig. 3). In both years of the experiment, it had inhibiting effect after day 7 whereas the strongest manifestation was observed fifteen days after the input. Some convergence between the values of herbicide treated beds and the controls was observed during the first year, which was probably due to the drought that occurred that year. Larger number in the controls compared to the treated beds was discovered during the second year of the study. This was due to the more even distribution of precipitations over the whole period of the study. In 2007, there were periods with alternating higher and lower precipitations, which decreased the total number of the cellulose decomposing bacteria in both treated beds and controls.

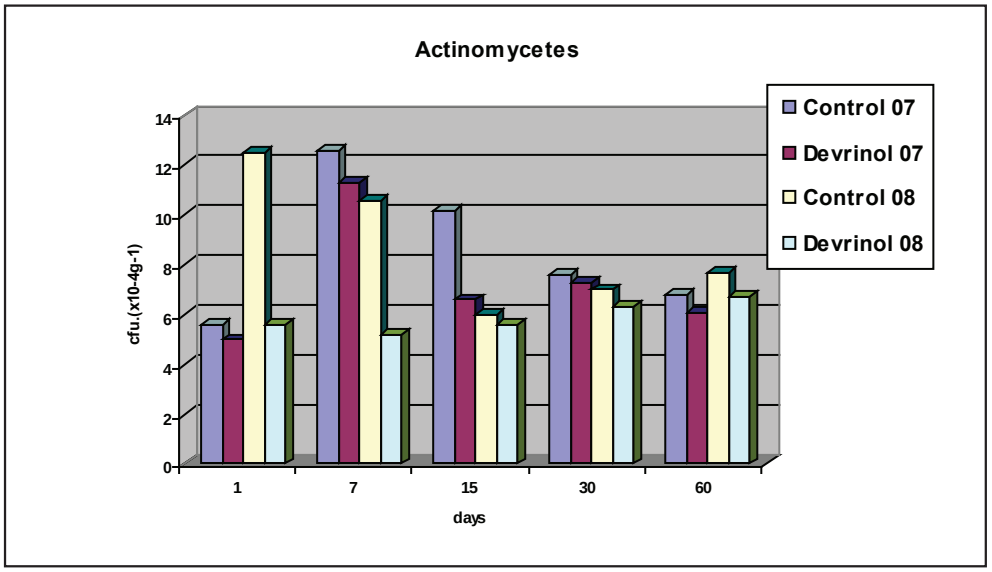

Fig. 4: Number of actinomycetes

Actinomycetes are widely spread in the soil and they are present in greater numbers than spore-forming bacteria (see Fig. 4).

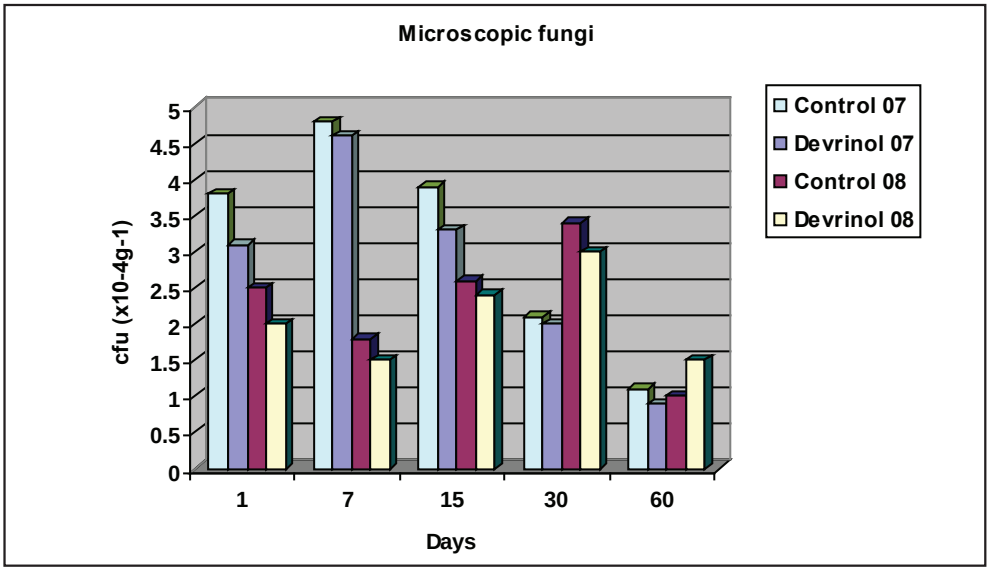

Fig. 5: Number of microscopic fungi

The analysis of the presented data shows that the reduction in the number of actinomycetes is overcome on the thirtieth day. The soil moisture as a factor is sufficient to overcoming the negative effect of the herbicide. As a result, the processes of mineralization are accelerated and access to nutrients in the soil is provided.

Along with other groups of microorganisms, microscopic fungi take part in the decomposition of various remains and in the synthesis of organic compounds. The effect of napropamide on this physiological group is shown in Fig. 5. The seasonal variations both in the treated beds and the controls are very clear up to day 60. At the last reading, the quantitative manifestation is similar in both parts, probably again due to the drought, which has negative impact on them.

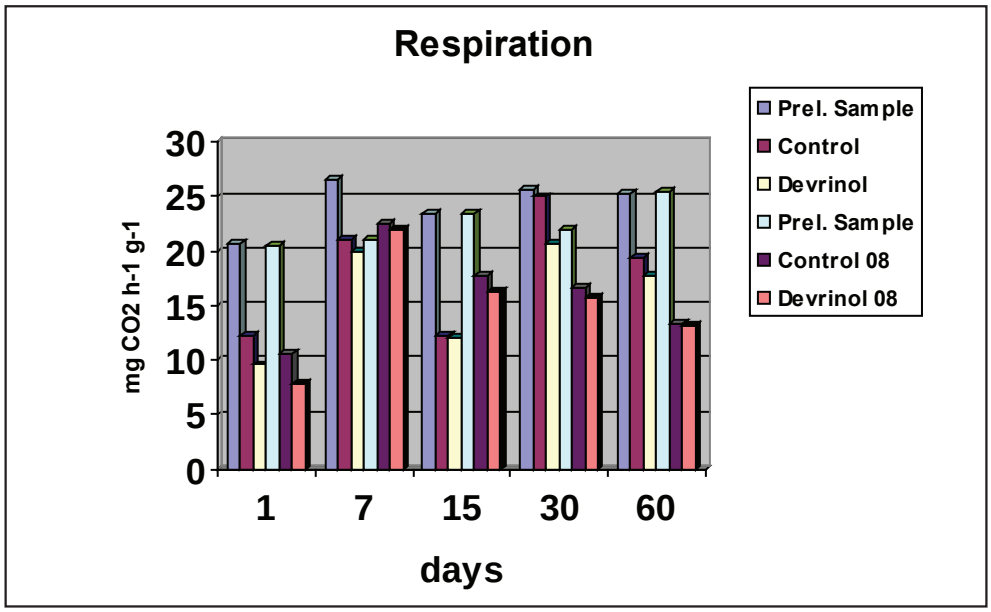

Fig. 6: Total biological activity in the soil

This study shows the impact of Devrinol F on the total biological activity in the soil (Fig 6). It reveals that the herbicide causes troubles in the metabolism but to an insignificant extent. The largest reduction of CO_2 emissions was observed on day 1 after treatment, and up to day 60 this effect is overcome thank to the great adaptation capacity of microorganisms to different habitats.

CONCLUSION

The study conducted to establish the effect of the soil herbicide Devrinol 4F on some essential physiological groups of microorganisms has revealed that the herbicide inhibits these groups. The results we obtained confirm the conclusions drawn from other studies that a major part of the active substance in the herbicide is decomposed within 20 days. Thirty days after the treatment, the quantities of microorganisms in the treated beds and the controls are insignificant.

The use of the herbicide Devrinol 4F in the production of tobacco seedlings inhibits to an insignificant extent some essential physiological groups of microorganisms. The negative effect is overcome sixty days after treatment and the quantities of microorganisms in the treated material and in the controls are similar.

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BALANCE OF TOBACCO IN THE REPUBLIC OF MACEDONIA

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ABSTRACT

In this paper, the authors use mainly statistical data on the quantity of produced, consumed, exported and imported tobacco in the period 2001-2010. According to these data, the authors make the balance of supply and demand of tobacco. By definition, the balance is the difference between the demand and supply of tobacco and tobacco products. The balance is shown in material and financial terms.

Generally, tobacco supply on Macedonian market is greater than the demand. However, the authors also reported that in certain periods, the demand is greater than the available amount of tobacco on the market. The analysis shows negative values for the balance in the research period, i.e. the value of tobacco demand was 158,2% higher compared to the value of tobacco and tobacco products offered on Macedonian market.

Key words: demand, supply, balance

БИЛАНС НА ТУТУНОТ ВО РЕПУБЛИКА МАКЕДОНИЈА

Во трудот се користени главно статистички податоци за количеството на произведен, потрошен, извезен и увезен тутун во периодот од 2001 до 2010 година. Врз база на овие податоци авторите прават биланси на понудата и побарувачката на тутун. По дефиниција, билансот е разликата помеѓу понудата и побарувачката на тутун и производи од тутун. Билансот го прикажуваат во материјален и финансиски израз.

Авторите утврдиле дека, главно, на пазарот на тутун во Република Македонија се нуди поголемо количество отколку што се бара, но и дека во одредени периоди има поголема побарувачка од расположливото количество.

Анализата на билансот во вредносен израз покажала дека во целиот истражуван период билансот е негативен, т.е. вредноста на побарувачката на тутун е за 158,2% повисока од вредноста на тутунот и производите од тутун што се нудат на македонскиот пазар.

Клучни зборови: побарувачка, понудувачка, била

INTRODUCTION

The conventional use of tobacco is a specific business for many stakeholders, households for primary production of tobacco, purchase companies, enterprises for export/import of tobacco as well as enterprises for cigarette production. Considering these facts, it means that tobacco is a culture and a source of existence for a great number of inhabitants in Macedonia. The activities related to tobacco do not have consistent intensity during one year or in the course of many consecutive years. They depend on the demand, supply of tobacco and tobacco products.

In practice, it is very rare case or almost impossible for equality between the supply and the demand i.e. to maintain balance. When the supply is greater than the demand, there is a positive balance of tobacco and vice versa.

The purpose of this paper is to determine the dynamics of supply and demand of tobacco and tobacco products during a ten year period.

MATERIAL AND METHOD

The supply of tobacco and tobacco products consist of the amount of produced tobacco and the amount of imported tobacco while the demand itself is a sum of domestic consumption and the amount of exported tobacco and tobacco products. For all balance elements of tobacco, it was used statistical data published by the State Statistical Office of the Republic of Macedonia for the period 2001-2010. It was also used Statistical Yearbook and Statistical Review: Foreign trade.

The balance of supply and demand of tobacco, as well as of tobacco products was calculated by the following formula:

$$Eq = (Pr + Im) - (CD + Ex)$$

Pr – production of tobacco (purchased tobacco),

Im – tobacco import and tobacco products,

CD – domestic consumption of tobacco and tobacco products,

Ex – tobacco export and tobacco products.

Original data were used from the State Statistical Office for the quantity and value of tobacco, as well as for tobacco export/import. The domestic consumption was calculated according to the following statistical data; annual expenditure for personal consumption of tobacco per household, number of households, average price of cigarettes with filter of I quality group, and technological normative for number of 1 kg cigarettes of oriental tobacco.

RESULTS AND DISCUSSION

1. Quantitative expression of tobacco balance

It is noteworthy to be mentioned that tobacco balance is relatively difficult. The difficulties appear in consequence of the purchasing companies which do not sell (or they cannot at all) the wholly quantity in the same year, but rather they leave some reserves. The state keeps a certain amount of it as commodity stocks, and after several years, it is available on the market. Furthermore, a certain amount of the import is exported as a finished product, while some amount of raw tobacco cannot be found on the market, i.e. it is not statistically registered, but consumed in the households. Presumably, a small part of tobacco is illegally exported outside the country. All of these facts create problems for the balance of tobacco.

Table 1 - Dynamics of tobacco balance according to quantities

Year	Tobacco quantity, tons		
	Supply	Demand	Balance
2001	25,886	18,014	7,872
2002	25,705	19,880	5,825
2003	26,761	25,398	1,363
2004	26,088	19,249	6,839
2005	28,185	25,357	2,828
2006	28,714	33,414	-4,700
2007	25,605	27,551	-1,946
2008	21,941	24,645	-2,704
2009	27,774	20,007	7,767
2010	35,628	21,804	13,824
Average	27,229	23,532	3,697

Source: Statistical Yearbook of R. Macedonia 2001-2010

It is our hope that in the future, all of the subjects involved in tobacco sector will behave more rationally and that the variations on tobacco market will not be as great as in the past (Figure 1).

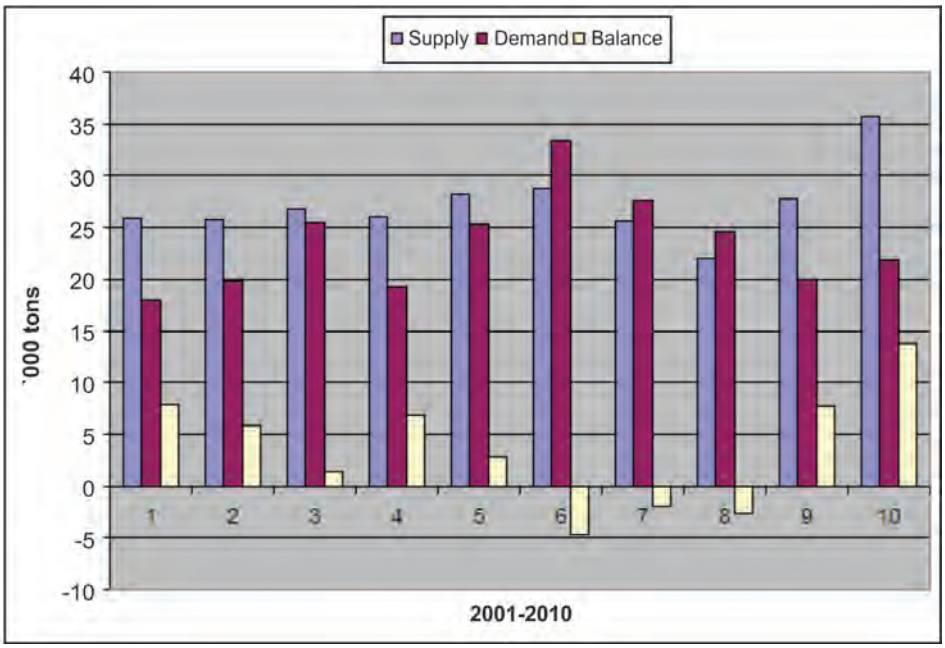

Figure 1- Quantitative balance of supply and demand

However, mostly during the analyzed period, Macedonia offers greater quantity than needed. The data show that the quantity was in highest surplus in 2010 (Table 1). This is due to the fact that in 2010, the supply was the highest (above 35, 6 thousand of tons), with the largest production of (above 30 thousand of tons) and with the largest import of (above 5, 3 thousand of tons). The largest deficit or rather the highest negative balance was recorded in 2006. In that year, the demand was 16, 4% higher than the supply, due to the impact of the export. That year, Macedonia recorded an export of 32 thousand of tons.

The downward trend in domestic consumption continues to decrease (Filiposki B., 2010). If the export and production continue to decrease, while supply increases, in 2011 and even after, the problems will become tough on the Macedonian market of tobacco.

2. Financial expression of tobacco balance

The analysis of data (Table 2) for the financial expression of tobacco balance reveals that during the period of research, the value of demand was higher than the value of supply. This is due to tobacco higher use and thus, economic value in the process of fabrication. The tobacco prepared for export has also higher quality and economic value.

Table 2 - Dynamics of tobacco balance based on value

Year	Tobacco value, 000 denars		
	Supply	Demand	Balance
2001	3,550,603	9,005,803	- 5,455,200
2002	3,602,602	10,029,740	- 6,427,138
2003	2,753,079	9,335,392	- 6,582,313
2004	3,403,461	8,008,989	- 4,605,528
2005	3,984,102	9,488,516	- 5,504,414
2006	3,858,160	11,767,648	- 7,909,488
2007	3,134,024	10,782,429	- 7,648,405
2008	3,321,548	11,937,082	- 8,615,533
2009	5,304,069	10,830,448	-5,526,379
2010	6,578,435	10,781,443	-4,203,008
Average	3,949,008	10,196,749	-6,247,741

Source: Statistical Yearbook of R. Macedonia 2001-2010

The culmination of difference was reached in 2008, but after, it started to decrease (Figure 2). On the one hand, it was primarily due to decreased export prices, and on the other hand, because of the decline by 17% in annual expenditure for personal consumption of tobacco per household.

The fact that Macedonia is a good producer of tobacco is a base for further expansion of exports to the old and new markets.

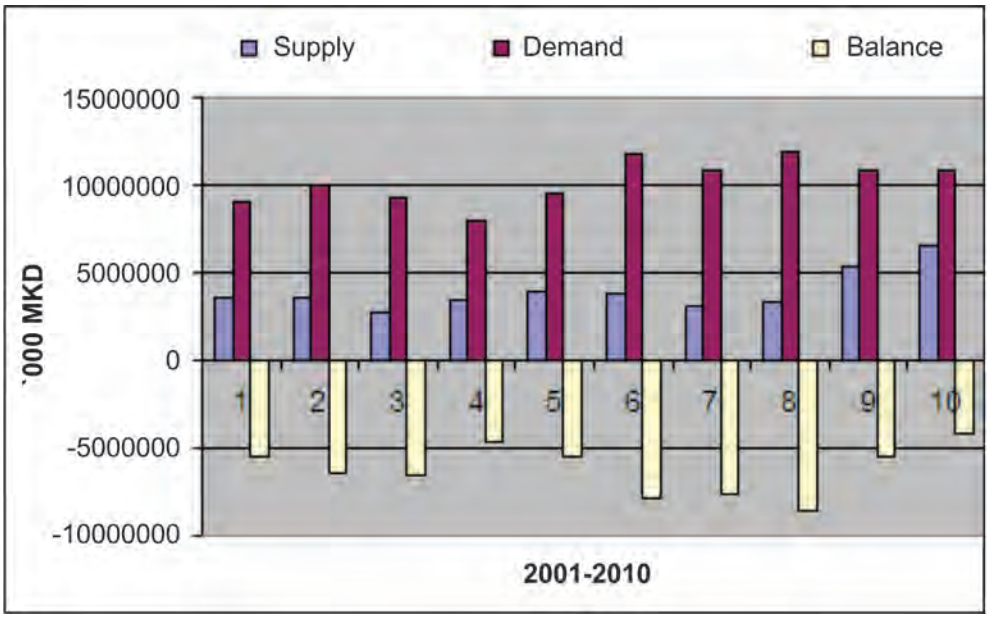

Figure 2 – Financial balance of supply and demand

CONCLUSIONS

According to the results of the research on the supply and demand of tobacco and tobacco products, we can draw the following conclusions:

• R. Macedonia has an extra quantity of tobacco in the domestic market

• R. Macedonia is continuously present on the international market of oriental tobacco in terms of the export. However, while the quantity is decreasing, the price is relatively increasing.

• Apart from exporting, R. Macedonia imports mainly Virginia tobacco types, and the quantities are in slight increase.

• During the ten year period (2001-2010), a downward trend was recorded in the domestic consumption.

• Due to the fact that the elements of demand (domestic consumption and export) in the process of processing are with added quality value, they are more economically valued on the market. For this reason, the pecuniary value has been always higher than the supply.

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