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Original Scientific Paper

MORPHOLOGICAL TRAITS OF SOME VIRGINIA TOBACCO VARIETIES AND LINES IN 2019 YEAR

Karolina Kocoska

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

ABSTRACT

Investigation included four foreign varieties: V-385 Ø, RxT, Hewessi 4, L. 17-66 and two domestic male sterile hybrid lines: V-112 CMS F_1 and V-97 CMS F1. The trial was set up using the method of randomized blocks in four replications. Therefore, the purpose of investigation of the Virginia varieties and lines was to present the characteristics of the middle belt insertions: length and width of the 5 th, 10th and 15th leaf in green condition, height of the plant with inflorescence and the number of leaves per plant. Measurements of the investigated varieties and lines were made during the flowering period. To obtain the significance of these investigations, the results were statistically processed and tested with LSD test. The best results were obtained with the lines V-112 CMS F_1 and V-97 CMS F_1, with statistical significance of 1% compared to the check in all examined traits.

Key words: *tobacco, leaf, length, width, varieties.*

МОРФОЛОШКИ ОСОБИНИ КАЈ НЕКОИ ВИРЏИНИСКИ СОРТИ И ЛИНИИ ТУТУН ВО 2019 ГОДИНА

Во испитувањата беа земени четири странски сорти: V-385 Ø, R×T, Hewessi 4, и L. 17-66 како и две домашни машкостерилни хибридни линии креации на НИТ-Прилеп V-112 ЦМС F_1 и V-97 ЦМС F_1. Истржувањата беа направени на опит по методот случаен блок систем во четири повторувања. Целта во испитувањата на вирџиниските сорти и линии ни беше да ги претставиме карактеристиките на инсерциите од средниот појас: должна и широчина на 5 ти, 10 ти и 15 ти лист во зелена состојба, висина на растението со соцветие и број на листови по растение. Мерењата на испитуваните сорти и линии беа направени во периодот на цветање. За да се добие значајноста на овие испитувања добиените резултати беа и статистички обработени и тестирани со LSD тест. Во најголем дел од испитуваните својства најдобри резултати покажаа линиите V-112 ЦМС F_1 и V-97 ЦМС F_1 кои во сите испитувани својства имаа статистичка значајност за 1% во споредба со контролата.

Клучни зборови: тутун, лист, должина, ширина, сорти.

INTRODUCTION

In parallel with creating new varieties of prilep, yaka, djebel, basmak, otlya and burley tobacco types, Scientific Tobacco Institute - Prilep is actively working on creating new varieties of virginia tobacco type. The varieties of virginia tobacco type participate in Blend cigarettes at about 50-60%, depending on the recipe of the cigarette. Characteristic of this tobacco type is that it is cured in hot air (Flue-cured) and it is purchased in green and dry condition. North Macedonia offers very favorable conditions for the development of the large-leaf tobacco production, especially of virginia type, thanks to its climatic, pedological and hydrological characteristics. For years, producers have not produced this tobacco type, as a result of unregulated purchase of large-leaf tobacco by the tobacco companies. Miceski, Stojanoska, Risteski (2001) emphasize that in North Macedonia, the production of Virginia tobacco, from 1991 to 1996, ranged from 6% to 8% of the total tobacco production. The same authors emphasize that there are favorable conditions for growing this tobacco type and for its farm production.

The main orientation in breeding of this tobacco type is creation of male sterile hybrid varieties, which in most properties have proven to be better than the fertile varieties. Before the registration in the State Commission for Variety Testing, the varieties (lines) that proved to be good (as a result of the breeding) are previously examined in comparison with other recognized varieties in comparative trials. According to the obtained results, it can be seen that the new varieties have already been created and they will be reported for recognition in the State Commission for Variety Testing, if there are conditions for proper curing of the tobacco leaf.

MATERIAL AND METHODS

The seedlings were produced in Scientific Tobacco Institute - Prilep. Same cultural practices were applied for the examined varieties. Sowing of the seed material was performed with 3g of seed material per 10 m2. The trial was set up in the field of STI-Prilep on alluvial-colluvial type of soil, previously examined, prepared and fertilized with 300 kg/ha NPK fertilizers in a ratio of 8:22:20. Investigation included four foreign varieties as a check: fertile variety V-385 from Poland, RxT from the USA, Hewessi 4 from Hungary, L-17-66 from Australia and two domestic male sterile hybrid varieties (lines): V-112 CMS F1 and V-97 CMS F1 from North Macedonia, created in STI - Prilep. The trial was set up using the method of randomized blocks in four replications, with planting density 90×50 cm. During the growing period, two hoeing and one feeding with 26% KAN (3-4g/stalk) were performed. Also, several irrigations and treatments with protective products against diseases and pests were performed. Following measurements were made during the flowering period of the plants: length and width of the 5th, 10th and 15th leaf in green condition, height of the plant with inflorescence and number of leaves per plant. Morphological measurements were made on 10 plants, and the average values were tabulated. The obtained results were statistically processed using the method of analysis of variance and LSD test.(Najceska, 2002, Filiposki 2011).

RESULTS AND DISCUSSION

Morphological characteristics of the varieties

Data on the morphological measurements of the varieties are provided in Table 1. According to the data in Table 1, line V-112 CMS F_1 is characterized by the highes length of the 5th leaf (58 cm), and variety L-17-66 with the lowest length (41 cm). In the other varieties, this data ranges from 45.5 cm in R×T, to 56.9 cm in line V-97 CMS F_1. Line V-97 CMS F_1 is characterized by the highest width of the 5th leaf (39.60 cm) and variety L-17-66 by the lowest width (26.2 cm).

Table 1. Results of the morphological measurements (average values)

Variety (Line)	5th leaf		10th leaf		15 th leaf	
	length	width	length	width	length	width
V-385 Ø	46.4	26.5	61.1	32.3	54	26.3
R×T	45.5	33.7**	60.4	44.4**	57.2	33.8**
Hewessi 4	51.1*	32.8**	59.8	38.2**	55.8	31.6**
L-17-66	41	26.2	53.7	30.8	50.2	29.1
V-112 CMS F_1	58**	36.5**	71.8**	42.1**	63.2**	36.8**
V-97 CMS F1	56.9	39.60**	70.4**	43.0**	65.7**	37.4**

Length of 5th leaf	Length of 10th leaf	Length of 15th leaf
LSD 5% * = 3.98 cm	LSD 5% * = 3.02 cm	LSD 5% * = 4.85 cm
1% ** = 5.51 cm	1% ** = 4.18 cm	1% ** = 6.71cm
Width of 5th leaf	Width of 10th leaf	Width of 15th leaf
LSD 5% * = 3.05 cm	LSD 5% * = 2.13 cm	LSD 5% * = 3.65 +cm
1% ** = 4.23 cm	1% ** = 2.95 cm	1%** = 5.05++cm

In other varieties, the width of this leaf ranges from 26.5 cm in V-385, to 36.5 cm in line V-112 CMS F_1. Line V-112 CMS F_1 is characterized by the highest length of the 10th leaf (71.8 cm), and line L-17-66 by the lowest length (53.7 cm). In the other varieties the length of the 10th leaf ranges from 59.8 cm in Hewessi 4 to 70.4 in line V-97 CMS F_1. The lowest width of the 10th leaf is registered in variety V-385 (32.3 cm) and the highest (43.0 cm) in line V-97 CMS F_1. For the characteristic Length of the 5th and 10th leaf, the statistical significance at 1% level compared to the check was obtained in both lines, V-112 CMS F_1 and V-97 CMS F_1. For the characteristic Length of the 5th leaf, in Hewessi 4 was obtained statistical significance at 5% level, and for the Width of the 5th and 10th leaf, statistical significance at 1% level was obtained in the following varieties and lines: R×T, Hewessi 4, V-112 CMS F_1 and V-97 CMS F_1.

According to the obtained data, the hybrid lines have larger leaves dimensions compared to the other varieties in the trial, which will increase their yield. Hawks S.N. (1978) and Gornik R. (1973) reported that F1 hybrids in male sterile form are characterized by higher yields, higher resistance to some diseases, early maturation etc.

The highest length of the 15th leaf (65.7 cm) was observed in line V-97 CMS F_1 and the lowest (50.2 cm) in variety L-17-66. In other varieties, the length of the 15th leaf ranges from 69.2 cm in line V-112 CMS F_1, to 54 cm in check variety V-385. The highest width of the 15th leaf (37.4 cm) was observed in line V-97 CMS F1, and the lowest (26.3 cm) in check V-385. In the other varieties examined in this trial, the width of this leaf is in range of 36.8

cm in V-112 CMS F_1, up to 29.1 cm in variety L-17-66. Lines V-112 CMS F_1 and V-97 CMS F1 obtained statistical significance at 1% level, for the characteristic Length and width of the 15th leaf. Statistical significance at 1% level was shown in varieties R × T and Hewessi 4, for the characteristic Width of the 15th leaf.

Dražić et al. studied 13 genotypes of Virginia tobacco in Nova Pazova (Serbia) in 2011 and found that the average size of the middle-belt leaves was 64 cm length and 30 cm width. Kalamanda (2009) in Republika Srpska performed 3-year investigations with two Virginia tobacco varieties and reported that the middle belt leaf had 48.7 cm average length and 22.7 cm average width. According to the obtained data, the examined varieties and lines have a characteristic leaf size for this type of tobacco, especially the hybrid lines V-112 CMS F_1 and V-97 CMS F_1.

Table 2. Plant height and number of leaves
(average values)

Variety (Line)	Height of the stalk in cm	Number of leaves
V-385 Ø	190	26.4
R×T	162	30.2^{**}
Hewessi 4	154	26.2
L-17-66	168	24.6
V-112 CMS F_1	198	32^{**}
V-97 CMS F_1	201^{*}	31.8^{**}

	Height of the stalk		Number of leaves
LSD	$5\%^{*}$ = 8.53 cm	LSD	$5\%^{*}$ = 1.97 cm
	$1\%^{**}$ = 11.81 cm		$1\%^{**}$ = 2.72 cm

According to Table 2, the highest stalk height with inflorescence (201 cm) was measured in line V-97 CMS F1, and the lowest (154) in variety Hewessi 4. In other varieties, the average stalk hight ranges from 198 cm in line V-112 CMS F_1 to 162 cm in R × T. Devčić K. et al. (1982) reported that the height of the Croatian varieties H-10, H-31 and H-32 was 170 cm. According to Beljo (1996) and Uzunoski (1985), the Virginia tobacco belongs to the group of high tobaccos (about 200 cm), which require precisely determined agroecological conditions and cultural practices for their growth and development, including the specific method of drying (flue-curing).

On average, line V-112 CMS F_1 is characterized by the largest number of leaves per stalk (32), and variety L-17-66 by the lowest (24.6). In other varieties, the number of leaves ranges from 31.8 in line V-97 CMS F1, to 26.2 in variety Hewessi 4. Statistically significant differences at 1% level in height of the plant were recorded in lines V-112 CMS F1, V-97 CMS F1 and variety R×T, compared to the check V-385.

Growing period length (flowering)

The cycle from planting the tobacco seedlings to tobacco flowering stage is defined as a growing period, which is primarily conditioned by the genetic characteristics of the type and variety. Table 3 presents data on the length of the growing period in the examined varieties and lines in 3 phases: beginning of the flowering from transplanting date in days, 50% flowering and end of the flowering.

Table 3. Length of the growing period from 2019

Variety (line)	Beginning of the flowering from transplanting date in days	Difference		50% flowering from transplanting date in days	Difference		End of the flowering from transplanting date in days	Difference	
		Absolute	Relative		Absolute	Relative		Absolute	Relative
V-385 Ø	72	/	100.00	77	/	100.00	81	/	100.00
R×T	67	-5	93.05	72	-5	93.50	76	-5	93.82
Hewessi 4	67	-5	93.05	71	-6	92.21	75	-6	92.59
L-17-66	73	+1	101.38	77	0	100.00	81	0	100.00
V-112 CMS F_1	75	+3	104.16	79	+2	102.59	84	+3	103.70
V-97 CMS F1	74	+2	102.77	78	+1	101.30	81	0	100.00

Acording to the data above, it can be seen that the flowering first started in varieties R×T and Hewessi 4 (67 days) and the last in line V-112 CMS F_1 (75 days). In other varieties and lines, this data ranges from 72 days in check V-385, up to 74 days in line V-97 CMS F_1. Rubin (1971) concluded that the first flower that blooms is the central (top) flower of the inflorescence. Naumoski et al. (1977) reported that tobacco plant has already built 90% of its plant mass by the end of flowering. Line V-112 CMS F_1 is characterized by the longest growing period from the planting date to 50% flowering (79 days) and Hewessi 4 by the shortest (71 days). In other varieties and lines, this data ranges from 72 days in variety R×T, up to 78 days in line V-97 CMS F_1. The phase End of the flowering from transplanting date is the longest in line V-112 CMS F1 (84 days), and the shortest in variety Hewessi 4 (75 days). In other varieties and lines, this data ranges from 76 days in variety R×T, up to 81 days in line V-97 CMS F_1 and varieties V-385, L-17-66. Risteski and Kocoska (2014) in investigation of 7 domestic and foreign tobacco varieties and lines in the region of Prilep in 2010/2011 reported that the end of flowering occurs after 71.5 - 79 days. According to data above, we conclude that the hybrid lines compared to the foreign varieties (R×T and Hewessi 4), are characterized by longer growing period.

CONCLUSIONS

- Regarding the morphological traits, all varieties and lines included in this trial fit within the limits that apply to this type of tobacco.
- The highest average length of the 5th and 10th leaf was recorded in line V 112 CMS F_1. The length of the 5th leaf is 58 cm and the length of the10th leaf is 71.8 cm. The length of the 15th leaf is the highest in line V-97 (65.7 cm).
- The highest average width of the 5th, 10th and 15th leaf was recorded in line V 112 CMS F_1 and it is 39.60 cm, 43.0 cm and 37.4 cm respectively.
- The highest average height of the stalk was measured in line V-97 CMS F_1 (201 cm), and the lowest in variety Hewessi 4 (154 cm).

- The average number of leaves was the highest in line V-112 CMS F_1 (32), and the lowest in variety L-17-66 (24.6).
-The morphological measurements made in this trial lead to a conclusion that lines V-112 CMS F1 and V-97 CMS F_1 dominate over the other varieties.
- According to data obtained from the trial, we can conclude that the hybrid lines are characterized by longer growing period, compared to the foreign varieties (R × T and Hewessi 4).
- The results of the examined properties indicate the conclusion that the hybrid lines are characteristic for the Virginia type and that there are appropriate agroecological and climatic conditions for production of this tobacco type in our country.

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MANIFESTATION OF HETEROSIS AND INFLUENCE OF THE DIRECTION OF CROSSING UPON SOME CHARACTERS IN HYBRID COMBINATIONS OF BURLEY TOBACCO

Yovko Dyulgerski, Radka Bozhinova

Agricultural Academy, Tobacco and Tobacco Products Institute (TTPI), Markovo, Bulgaria
(e-mail:yovk_dulg@abv.bg)

ABSTRACT

The results of the study show that for the length of the vegetative period, heterosis phenomena are not essential for the selection of Burley tobacco. Regarding the size of the yield, the heterosis manifestations are also not strongly expressed. However, the heterosis phenomena are very strongly expressed in terms of the percentage of the first grade. Crossing direction is more significant on heterosis for size of yield, but not for length of vegetative period, and for percentage of first grade, crossing direction is critical. Burley 1344 variety has a pronounced combinative ability, participating as a parent component in the investigated hybrid combinations. In general, no positive relationship is observed between the occurrences of heterosis for yield size and those for first grade percentage. From the economic and selection point of view, Hybrid 1579 and Hybrid 1577 perform best, and also to some extent Hybrid 1573 and Hybrid 1573A.

Key words: tobacco, heterosis, vegetative period, first grade

МАНИФЕСТАЦИЈА НА ХЕТЕРОЗИСОТ И ВЛИЈАНИЕ НА НАСОКАТА НА ВРСКУВАЊЕ ВРЗ НЕКОИ КАРАКТЕРИСТИКИ КАЈ ХИБРИДНИТЕ КОМБИНАЦИИ НА БАРЛЕЈСКИ ТУТУНИ

Резултатите од студијата покажуваат дека за времетраењето на сезоната на растење, феномените на хетероза не се од суштинско значење за селекцијата на тутунот Бурлеј. Во однос на големината на приносот, манифестациите на хетерози исто така не се силно изразени. Сепак, феномените на хетероза се многу силно изразени во однос на процентот на прва класа. Насоката на вкрстување беше позначајна за хетерозата за големината на приносот, но не и за должината на сезоната на растење, а за процентот од прва класа, насоката на вкрстување беше критична. Сортата Burley 1344 има изразена комбинативна способност, учествувајќи како матична компонента во истражуваните хибридни комбинации. Општо земено, не беше забележана позитивна врска помеѓу појавата на хетероза за големината на приносот ио не и за процентот од прва класа. Од економска и селективна гледна точка, најдобри резултати покажаа Hybrid 1579 и Hybrid 1577, а исто така до одреден степен и Hybrid 1573 и Hybrid 1573A.

Клучни зборови: тутун, хетерозис, вегетациски период, прва класа

INTRODUCTION

The dynamics and growing demands of the domestic and international markets for tobacco varieties constantly pose new tasks for tobacco selection (Nikolova et al., 2008; Kocoska, 2018; Kurt et al., 2020; Nikolov et al., 2022; Aleksoski et al., 2023; Tsaliki, 2023). Heterosis selection in large-leaf tobacco provides very good opportunities for creating new varieties and helps to intensify the selection process (Shabanov and Tomov, 1989; Butorac, 2000; Korubin – Aleksoska, 2016; Manolov, 2000; Gixhari and Canllari, 2004; Pearce et al., 2019; Kinay and Kurt, 2022; Aleksoski, 2023).

Heterosis is a biological phenomenon that is observed in first-generation hybrids and is expressed in an increase in the power, viability and productivity of hybrids compared to those of the original parental forms (Yankulov, 1996). When the value of the trait increases in hybrids compared to the parental forms, the heterosis is called positive, and when it decreases, it is called negative (Delchev et al., 1996). Heterosis is used only in the first hybrid generation because it is most pronounced and because the plants are phenotypically uniform (Patel et al., 2012; Schnable and Spinger, 2013).

Most often, the heterosis effect mainly affects te increase in vegetative mass and growth rate (Tchinchev, 1984; Pearce et al., 2014). For large-leaved tobacco varieties, a rapid initial rate of growth and development is very important in order to shorten the long rosette phase when tobacco is most vulnerable (Pophristev, 1977; Prasannasimharao, 1995; Xu and Zhu, 1999).

While the heterosis effect with respect to the size of the yield in tobacco is the subject of a large number of studies, there are relatively few for the length of the vegetative period, and for the quality shown by the percentage, the grades are downright scarce (Dyulgerski, 2011; Aleksoski, 2022; Sufan et al., 2023).

Literature data on the influence of the crossing direction on the manifestations of the heterosis effect in tobacco are few and quite contradictory (Vasilev and Dimitrova, 2010; Dimitrova and Vassilev, 2010; Ramanarao et al., 1993; Kara and Esendae, 1995), which necessitates new studies on the subject.

The purpose of the present study is to study heterosis manifestations and to establish the influence of the direction of crossing on them in terms of the most important economic and productive indicators: length of the vegetative period, yield size and the percentage of grades in hybrid combinations of Burley tobacco.

MATERIAL AND METHODS

The experimental work is carried out during the period 2014-2020 at the experimental field of TTPI – Markovo, Bulgaria. Twenty hybrid combinations created by us in F_1 tobaccos from the Burley cultivar group are subjected to research. Each hybrid combination is represented by its direct and reverse cross - the variant that is a maternal component in one is a paternal component in the reciprocal. Both established indigeneous and introduced varieties and promising lines selected by us are involved as parental components. For all variants, the length of the vegetative period, the size of dry tobacco yield per hectare and the percentage of the first grade are recorded.

An analysis of variance (ANOVA) is performed on the obtained data. Arithmetic mean (x), arithmetic mean error (Sx %) are calculated in relation to the studied indicators. Hypothetical and true heterosis are determined according to Omarov (1975). Hypothetical heterosis is determined relative to the average value of the two parents, and the real one - relative to the value of the better parent (Omarov, 1975).

RESULTS AND DISCUSSION

Regarding the length of the vegetative period, negative heterosis is desired, as it is associated with a shorter one. The obtained results for heterosis manifestations for the length of the vegetation period show that for all hybrid combinations, the values of true heterosis are lower than those of the hypothetical one (Table 1). In this case, the differences in values between the two indicators are not significant. True negative heterosis with significant values is obtained in three out of all twenty crosses studied, and it is on the border of significance, being most strongly expressed in Hybrid 1577 (B 1344 x Ky 17). In cases where Burley1344 variety is used as the parent component, the heterosis effect has higher values. Positive heterosis is observed in one third of the studied crosses, which, however, is extremely undesirable in this case.

Table 1. Manifestations of heterosis in relation to the length of the vegetative period

Parents / Crosses	P_1 x±Sx%	P_2 x±Sx%	F_1 x±Sx%	HP hipothetical %	HP real %
Hybrid 1571 (L 1410 x B 1344)	76,7±0,27	74,5±0,26	71,7±0,25	-5,2	-3,8
Hybrid 1571 A (B 1344 x L 1410)	74,5±0,26	76,7±0,27	72,3 ±0,25	-4,4	-3
Hybrid 1572 (L 1410 x Ky 17)	76,7±0,27	77,7±0,27	79,5 ±0,28	3	3,7
Hybrid 1572A (Ky 17 x L 1410)	77,7±0,27	76,7±0,27	78,3±0,27	1,4	2,1
Hybrid 1573 (L 1349 x B 1344)	76,5±0,27	74,5±0,26	72,7±0,25	-3,7	-2,4
Hybrid 1573A (B 1344 x L 1349)	74,5±0,26	76,5±0,27	70,5±0,25	-6,6	-5,4
Hybrid 1574 (L 1349 x Tn 90)	76,5±0,27	80,3±0,28	80,3±0,28	1,2	0
Hybrid 1574A (Tn 90 x L1349)	80,3±0,28	76,5±0,27	81,0 ±0,28	3,3	5,9
Hybrid 1575 (L1349 x Tn 86)	76,5±0,27	76,3±0,27	75,5±0,26	1,2	-1
Hybrid 1575A (Tn 86 x L 1349)	76,3±0,27	76,5±0,27	77,7±0,27	1,7	1,8
Hybrid 1576 (L 1399 x Ky 907)	75,7±0,26	79,5±0,28	78,7±0,27	1,4	4
Hybrid 1576A (Ky 907 x L 1399)	79,5±0,28	75,7±0,26	79,3±0,28	2,2	4,8
Hybrid 1577 (B 1344 x Ky 17)	74,5±0,26	77,7±0,27	69,7±0,24	-8,4	-6,4
Hybrid 1577A (Ky 17 x B 1344)	77,7±0,27	74,5±0,26	70,5±0,25	-7,4	-5,4
Hybrid 1578 (L 1409 x Ky 17)	78,3±0,27	77,7±0,27	78,0 ±0,27	0	-0,4
Hybrid 1578A (Ky 17 x L 1409)	77,7±0,27	78,3±0,27	77,5±0,27	-0,6	-0,3
Hybrid 1579 (Tn 90 x B 1344)	80,3±0,28	74,5±0,26	71,3±0,25	-7,9	-4,3
Hybrid 1579A (B 1344 x Tn 90)	74,5±0,26	80,3±0,28	71,5±0,25	-7,6	-4
Hybrid 1580 (L 1322 x Tn 90)	77,3±0,27	80,3±0,28	76,7 ±0,26	-2,7	-0,8
Hybrid 1580A (Tn 90 x L 1322)	80,3±0,28	77,3±0,27	78,3±0,27	-0,6	1,3

Regarding this indicator, the heterosis phenomena are weakly manifested and are not essential for the selection of Burley tobacco. Influence of the crossing direction on the manifestation of heterosis with regard to this indicator is not observed.The obtained results for heterosis manifestations for the size of the yield show that for all hybrid combinations, the values of true heterosis are lower than those of the hypothetical one (Table 2). In this case, however, the differences in values between the two indicators are significant. True heterosis with significant values was obtained in three out of all twenty crosses tested. It is most pronounced in Hybrid 1579 (Tn 90 x B 1344). And in this case, the heterosis effect has higher values when the Burley 1344 variety is used as the parent component. The heterosis phenomena are not strongly manifested and are not essential for the size of the yield in the studied crosses.

An influence of the crossing direction with regard to this indicator is observed in a greater part of the hybrid pairs and was of greater importance than the length of the vegetative period.

Table 2. Manifestations of heterosis in terms of yield

Parents / Crosses	P_1 x±Sx%	P_2 x±Sx%	F_1 x±Sx%	HP hipothetical %	HP real %
Hybrid 1571 (L 1410 x B 1344)	284,3±1,59	338,7±1,90	346,3±1,93	11,2	2,2
Hybrid 1571 A (B 1344 x L 1410)	338,7±1,90	284,3±1,59	357,7±2,00	14,8	5,6
Hybrid 1572 (L 1410 x Ky 17)	284,3±1,59	271,3±1,52	287,5±1,61	3,6	1,1
Hybrid 1572A (Ky 17 x L 1410)	271,3±1,52	284,3±1,59	293,3±1,64	5,6	3,2
Hybrid 1573 (L 1349 x B 1344)	296,3±1,66	338,7±1,90	360,3±2,02	13,5	6,4
Hybrid 1573A (B 1344 x L 1349)	338,7±1,90	296,3±1,66	342,7±1,92	7,9	1,2
Hybrid 1574 (L 1349 x Tn 90)	296,3±1,66	307,0±1,72	294,7±1,65	-2,3	-4
Hybrid 1574A (Tn 90 x L1349)	307,0±1,72	296,3±1,66	317,3±1,78	5,2	3,4
Hybrid 1575 (L1349 x Tn 86)	296,3±1,66	292,5±1,64	302,7±1,70	2,8	2,2
Hybrid 1575A (Tn 86 x L 1349)	292,5±1,64	296,3±1,66	298,5±1,67	1,4	0,7
Hybrid 1576 (L 1399 x Ky 907)	288,7±1,62	302,3±1,69	290,3±1,63	-1,8	-4
Hybrid 1576A (Ky 907 x L 1399)	302,3±1,69	288,7±1,62	306,3±1,72	3,7	1,3
Hybrid 1577 (B 1344 x Ky 17)	338,7±1,90	271,3±1,52	353,5±1,98	15,9	4,4
Hybrid 1577A (Ky 17 x B 1344)	271,3±1,52	338,7±1,90	340,7±1,91	11,7	0,6
Hybrid 1578 (L 1409 x Ky 17)	281,5±1,57	271,3±1,52	283,7±1,59	2,6	0,8
Hybrid 1578A (Ky 17 x L 1409)	271,3±1,52	281,5±1,57	286,3±1,60	3,6	1,7
Hybrid 1579 (Tn 90 x B 1344)	307,0±1,72	338,7±1,90	368,7 ±2,06	14,2	8,9
Hybrid 1579A (B 1344 x Tn 90)	338,7±1,90	307,0±1,72	343,0±1,92	6,2	1,3
Hybrid 1580 (L 1322 x Tn 90)	314,7±1,76	307,0±1,72	308,7±1,73	-0,7	-1,9
Hybrid 1580A (Tn 90 x L 1322)	307,0±1,72	314,7±1,76	319,3±1,79	2,7	1,5

The obtained results for heterosis manifestations for the percentage of the first drade show that for all hybrid combinations, the values of the true heterosis are lower than those of the hypothetical (Table 3). In this case, the differences in values between the two indicators are very pronounced. In all hybrid combinations, in contrast to the length of the vegetative period and the size of the yield, a highly pronounced positive heterosis is established. It is especially strongly expressed in Hybrid 1576 and especially Hybrid 1579, where the heterosis exceeds 40%, which is very favorable.

In contrast to the other studied parameters, in this case a strong influence of the crossing direction is observed, and this is found in all studied hybrid combinations.

Table 3. Manifestations of heterosis in terms of percentage of the first grade

Parents / Crosses	P_1 x±Sx%	P_2 x±Sx%	F_1 x±Sx%	HP hipothetical %	HP real %
Hybrid 1571 (L 1410 x B 1344)	26±1,25	33±1,58	36±1,72	22	9,1
Hybrid 1571 A (B 1344 x L 1410)	33±1,58	26±1,25	43±2,06	45,8	30,3
Hybrid 1572 (L 1410 x Ky 17)	26±1,25	24±1,15	33±1,58	32	26,9
Hybrid 1572A (Ky 17 x L 1410)	24±1,15	26±1,25	29±1,39	16	11,5
Hybrid 1573 (L 1349 x B 1344)	28±1,34	33±1,58	40±0,25	31,1	21,2
Hybrid 1573A (B 1344 x L 1349)	33±1,58	28±1,34	37±1,78	21,3	12,1
Hybrid 1574 (L 1349 x Tn 90)	28±1,34	25±1,20	32±1,54	12,3	14,3
Hybrid 1574A (Tn 90 x L1349)	25±1,20	28±1,34	35±1,68	22,8	25
Hybrid 1575 (L1349 x Tn 86)	28±1,34	23±1,10	36±1,72	41,2	28,5
Hybrid 1575A (Tn 86 x L 1349)	23±1,10	28±1,34	34±1,63	33,3	21,4
Hybrid 1576 (L 1399 x Ky 907)	27±1,30	23±1,10	38±1,82	52	40,7
Hybrid 1576A (Ky 907 x L 1399)	23±1,10	27±1,30	30±1,44	20	11,1
Hybrid 1577 (B1344 x Ky 17)	33±1,58	24±1,15	44±1,11	54,4	33,3
Hybrid 1577A (Ky 17 x B 1344)	24±1,15	33±1,58	42±2,02	47,4	27,3
Hybrid 1578 (L 1409 x Ky 17)	31±1,49	24±1,15	34±1,63	23,6	9,7
Hybrid 1578A (Ky 17 x L 1409)	24±1,15	31±1,49	35±1,68	27,3	12,9
Hybrid 1579 (Tn 90 x B 1344)	25±1,20	33±1,58	47±2,26	62	42,4
Hybrid 1579A (B 1344 x Tn 90)	33±1,58	25±1,20	39±1,87	34,5	18,2
Hybrid 1580 (L 1322 x Tn 90)	30±1,44	25±1,20	32 ±1,54	16,4	6,7
Hybrid 1580A (Tn 90 x L 1322)	25±1,20	30±1,44	34±1,63	23,6	13,3

With the exception of Hybrid 1479 and partial hybrids with the numbers: H 1571A and H 1573, no positive correlation is observed between the manifestations of heterosis for the size of the yield and those for the percentage of the first grade. On the contrary, in most of the crosses, the manifestations of pronounced positive heterosis in the percentage of grades are related to the absence of such for the size of the yield. This is particularly manifested in Hybrid 1576, where, on the one hand, the most pronounced positive heterosis is observed for the percentage of the first grade, and even a negative one for the size of the yield. However, this relationship is not so strong.

CONCLUSIONS

In the studied hybrid combinations of Burley tobacco, the heterosis phenomena regarding the length of the vegetative period are not strongly expressed and are not essential for the selection. Crossing direction has almost no influence on the manifestations of heterosis effect in relation to the length of vegetative period.

In the studied hybrid combinations of Burley tobacco, the heterosis in the size of the yield is not strongly manifested and is not essential for selection and practice. The direction of crossing is more pronounced in the heterosis phenomena for the size of the yield than in the length of the vegetative period, but due to its weak manifestations it is also not of essential importance.

At the percentage of the first grade, the manifestations of heterosis are very pronounced. In this case, however, the direction of crossing is of essential importance. No positive correlation is observed between the heterosis manifestations for size of yield and those for percentage of first grade. In most hybrid combinations, it is rather negative, and this is especially strongly manifested in Hybrid 1576.

The variety Burley 1344 shows a pronounced combinative ability when it participates as a parent component in the investigated hybrid combinations.

As a result of the research, it was established that Hybrid 1579 and Hybrid 1577 are the variants with the greatest economic and selection value.

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Original Scientific Paper

POPULATION DYNAMICS OF NOCTUIDAE FAMILY- USING LIGHT TRAP

Vesna Krsteska[1], Stanislava Lazarevska [2], Biljana Jordanoska - Shishkoska[1], Kristina Hristovska[1], Marjana Gluseska[1]

[1]UKLO Scientific tobacco institute -Prilep,
[2]UKIM Faculty of agricultural science and food -Skopje
e-mail: vesna.krsteska@uklo.edu.mk

ABSTRACT

Helicoverpa armigera, Agrotis segetum and *Agrotis ipsilon* are significant agricultural pests on wide range of crops worldwide including tobacco. The studies were carried out on Experimental tobacco field in Prilep using light trap from beginning of March to the end of October 2020-2022.

In 2020 a total of 76 adults of *H. armigera*, 80 of *A. segetum* and 14 of *A. ipsilon* were caught, 74 moths of *H. armigera*, 45 of *A. segetum* and 25 adults of *A. ipsilon* in 2021, and 68 adults of *H. armigera*, 72 of *A. segetum* and 18 of *A. ipsilon* in 2022.

The maximum flight of *H. armigera* was during August 2020-2022. The maximum flight of *A. segetum* was from the third decade of May to the first decade of June and in the first and second decade of August 2020-2022. The maximum flight of *A. ipsilon* was in the first and second decade of August 2020-2022.

Effective monitoring is crucial for understanding population dynamics of pests, identifying outbreak patterns and implementing timely pest control measures. Long-term data collection capabilities make the light trap an important component of integrated pest management programs.

Key words: light trap, *H. armigera, A. segetum, A. ipsilon*

ДИНАМИКА НА ПОПУЛАЦИЈАТА ОД ФАМИЛИЈАТА NOCTUIDAE - СО УПОТРЕБА НА СВЕТЛОСНА ЛОВИЛКА

Helicoverpa armigera, Agrotis segetum и *Agrotis* ipsilon се значајни земјоделски штетници на широк спектар на култури ширум светот, вклучувајќи го и тутунот. Проучувањата беа изведени во експерименталното тутунско поле во Прилеп со употреба на светлосна ловилка од почетокот на март до крајот на октомври 2020-2022 година.

Во 2020 година беа фатени вкупно 76 возрасни од *H. armigera*, 80 од *A. segetum* и 14 од *A. ipsilon*, 74 молци од *H. armigera*, 45 од *A. segetum* и 25 возрасни од *A. ipsilon* во 2021 година, 68 имага од *H. armigera*, 72 од *A. segetum* и 18 од *A. ipsilon* во 2022 година.

Максималниот лет на *H. armigera* беше во текот на август 2020-2022 година. Максималниот лет на *A. segetum* беше од третата деценија на мај до првата деценија на јуни и во првата и втората декада на август 2020-2022 година. Максималниот лет на *A. ipsilon* беше во првата и втората декада на август 2020-2022 година.

Ефективното следење е од клучно значење за разбирање на динамиката на популацијата на штетниците, идентификување на моделите на појава и спроведување навремени мерки за контрола на штетниците. Долгорочните можности за собирање податоци ја прават светлосната стапица важна компонента на интегралните програми за управување со штетници.

Клучни зборови: светлосна ловилка, *H. armigera, A. segetum, A. ipsilon*

INTRODUCTION

Helicoverpa armigera Hübner - tobacco bollworm, is a pest of great agricultural importance, due to its polyphagy - wide host range, high reproductive potential and ability to migrate long distances.

Higher temperature and precipitation in spring and high temperatures in summer have a favorable effect on mass reproduction of this species (Vasilev et al. 1996).

Larvae of *H. armigera* successfully develop on tobacco plants where they cause economically significant damage. At the beginning of the vegetation, they feed on the young leaves making irregular holes. Other generations cause damage to the top leaves, flower buds, flowers, seed capsules. They can break through the stem and the top of the plant can break. Larvae cause great damage and loss of tobacco seeds (Krsteska, 2022, 2021, 2019, 2016, 2015, 2007).

Agrotis segetum Denis & Schiffermüller- common cutworm or winter is an economically important polyphagous pest of arable land, it is one of the most widespread cut-worm especially in regions with a temperate and continental climate (Vukasovic et al., 1962, Camprag 1973).

It is the most dangerous and widespread pest of arable land. For mass reproduction, a warm spring and summer with poor rainfall and a long warm and moderately humid autumn have a favorable effect (Vasilev et al. 1996).

A. segetum caterpillars can cause significant damage to the tobacco crop. In the lower larval stages, the larvae cause small circular lesions on young tobacco leaves, often damaging them down to the lower epidermis. In the larger larval stage, fallen leaves, leaves partially buried in the soil are observed, and the attacked plants turn yellow due to damage to the conducting vessels, wither and dry. The caterpillars make small holes in the stem near the root neck, and even cut whole young tobacco plants at the base of the stem.

Agrotis ipsilon Hufnagel- black cutworm is distributed almost all over the world.

It is a hygrophilous species and reproduces massively in warm years with early spring and long autumn and abundant and evenly distributed rainfall throughout the growing season (Vasilev et al. 1996, Kolektiv autora,1983, Vukasovic et al.,1962). The species occurs in large numbers in localities with a high level of underground water, which are abundant with moisture in the dry season. It was found in fields which were flooded and hardened in early spring (Camprag, 1973).

It is especially harmful to late planted tobacco plants or to plants that are behind in development. Young caterpillars make holes to the tobacco leaves. Third-stage larvae hide shallowly in the ground or under leaves lying on the ground during the day, and are active at night or in cloudy and rainy weather. They damage the stalks, and the older caterpillars cut the plants at ground level, and tobacco plants die.

Adult of both sexes of these investigated pests can be captured in light trap.

MATERIAL AND METHODS

The studies were carried out during 2020-2022 on tobacco plantations in Prilep. The light trap is used to determine the flight and the number of the adults. The trap is a strong light source with a power of 250 W (Figure 1). The lamp is placed at a height of 4 m, and is lit at night and burns until sunrise. The "owlet moths" are active at night and they are attracted to light and captured through a funnel in a metal cage placed under the lamp. The light trap was used from March to the end of October.

Figure 1. Light trap

RESULTS AND DISCUSSION

The family Noctuidae is the largest family of the Lepidoptera order. They are commonly known as owlet moths, cutworms or armyworms. The moths are dark and the forewings are larger and narrower than the hindwings. The forewings are gray, brown, with kidney-shaped, round and wedge-shaped spot (which are often indistinct) and other species-specific patterns (crossbands or crosslines). The hindwings are lighter white or yellowish with or without outer marginal stripe. The head is small brown, the compound eyes are large, and the proboscis is well developed. The antennae are threadlike or feathery. The chest is large and the stomach is cylindrical.

They are active at night, which is why they got the name night owls. Mimicry is present in the species and they are difficult to notice in the environment in which they live.

The investigated Noctuid moths commonly active season from spring to fall. Polyphagous larvae cause damage to many crops and weeds. They have several generations per year.

The species *H. armigera* is known as the tobacco bollworm, the cotton/corn borer, etc. Common synonyms for this species are: *Heliothis obsoleta, Heliothis armigera, Chloridea armigera, Chloridea obsoleta.*

The adult has a large body with body color varying from dark greenish yellow, olive green, greyish to light brownish yellow. The species is characterized by pronounced sexual dimorphism females are always darker than males. In females, the front wings are grey-ash, with hints of reddish-black color, while in males, the front wings are lighter brownish-yellow, with a more pronounced kidney-shaped pattern. The hindwings in both sexes are lighter than the forewings, with a broad dark brown outer marginal stripe (Figure 2).

Figure 2. *H. armigera*

The species is represented in large quantitative numbers on tobacco plantations every year. In 2020 a total of 76 adults were caught, 74 in 2021 and 68 on 2022. The *H. armigera* moths were determined in the second decade of June 2020 and in the third decade of June 2021-2022, until the second decade of October 2020/21, to second decade of September 2022. In 2020 the maximum flight was first and second decade of August, and then a greater number of moths were ascertained in the second decade of September. The maximum flight of the adults in 2021 is determined in the third decade of July and the first decade of August 2021, and in 2022 maximum flight of the adults is determined in the third decade of July to the first decade of September (Table 1, 2 and 3).

According to Tirelli (1955), *H. armigera* develop 2-3 generation per years. In Bulgaria there are 3 generations per year: from the end of April-May, then June-July, then the end of August-September (Camprag, 1973). They appear in large numbers in May-June and then during August (Kolektiv autora, 1983), May-June, July- August and September (Popov, 1962 cit. Vukasovi et al., 1962). During our investigations, the species was determined from the middle of June to the middle of October. Vasilev (1975) states that the butterflies are most active from July to September, when the conditions for the development of all developmental stages are most favorable.

Table 1. Quantitative representations of *H. armigera* in 2020
- method of light trap

Decade	Months							
	III	IV	V	VI	VII	VIII	IX	X
1	/	/	/	/	/	13	8	2
2	/	/	/	2	5	11	12	2
3	/	/	/	4	5	7	5	/
Total no /months	/	/	/	6	10	31	25	4
Total number	76							

Table 2. Quantitative representations of *H. armigera* in 2021
- method of light trap

Decade	Months							
	III	IV	V	VI	VII	VIII	IX	X
1	/	/	/	/	1	27	3	/
2	/	/	/	/	11.	5	1	1
3	/	/	/	2	21	2	/	/
Total no /months	/	/	/	2	33	34	4	1
Total number	74							

Table 3. Quantitative representations of H. armigera in 2022
- method of light trap

Decade	Months							
	III	IV	V	VI	VII	VIII	IX	X
1	/	/	/	/	1	11	12	/
2	/	/	/	/	3	12	6	/
3	/	/	/	1	15	7	/	/
Total no /months	/	/	/	1	19	30	18	
Total no /months		68						

The flight dynamics of this species coincides with the appearance of this species in pheromone traps (Krsteska, 2022, 2021). During the examination of the tobacco plantations for the presence of eggs and larvae of *H. armigera*, the presence of the species was determined after several days on the tobacco plants, which directed us to a more detailed daily monitoring of the pest in order to determine the time for the application of insecticides for the successful control of *H. armigera* on seed plantations.

Insecticidal treatment should be performed after emergence of the caterpillars and before they enter the seed capsules. Larvae during L5 and L6 burrow into the seed capsules and cause direct economic loss of seeds.

In 1972, the population of this species was much larger than in 2020 - 2022 with a total of 1900 specimens caught with the light trap. The first appearance of butterflies is in June, and the species is then represented from August to October, with the maximum development of the population in August with a total of 1328 butterflies caught (Todorovski, Vasilev, 1973). While in 1992 the species was represented from July to October with a total of 57 specimens, with two peaks in June and August (Vasilev et al., 1996). In 1993 and 1994 the catch was very poor with a maximum of 3 butterflies caught in 1993 and 17 specimens in 1994. In 1995, a total of 29 butterflies with two peaks were caught in June and August.

The species *A. segetum* is known as a common cutworm, winter owl. Known synonyms for this species are *Euxoa segetum, Scotia segetum*.

The moths have grey-brown to dark gray forewings, with characteristic spots that are often indistinct and distinct transverse dark lines. The hind wings are whitish, lighter in males, with dirty yellowish nervature (Figure 3).

Figure 3. *A. segetum*

In 2020 a total of 80 adults were caught, 72 in 2022, compared to 2021 when 45 adults were caught. The *A. segetum* moths were determined in the first decade of May 2020-2022, until the first decade of September 2021, second decade in 2022 and until the third decade of September 2020. The maximum flight was from the third decade of May to the first decade of June and in the first decade of

August 2020/21 and second decade of August (Table 4, 5 and 6).

Table 4. Quantitative representations of *A. segetum* in 2020 - method of light trap

Decade	Months							
	III	IV	V	VI	VII	VIII	IX	X
1	/	/	2	17	/	21	2	/
2	/	/	5	7	/	3	1	/
3	/	/	13	1	5	2	1	/
Total no /months	/	/	20	25	5	26	4	/
Total number	80							

Table 5. Quantitative representations of *A. segetum* in 2021 - method of light trap

Decade	Months							
	III	IV	V	VI	VII	VIII	IX	X
1	/	/	2	10	/	6	1	/
2	/	/	3	4	/	5	/	/
3	/	/	9	2	1	2	/	/
Total no /months	/	/	14	16	1	13	1	/
Total number	45							

Table 6. Quantitative representations of A. segetum in 2022 - method of light trap

Decade	Months							
	III	IV	V	VI	VII	VIII	IX	X
1	/	/	1	13	/	8	2	/
2	/	/	8	4	/	13	1	/
3	/	/	16	1	3	2	/	/
Total no /months	/	/	25	18	3	23	3	/
Total number	72							

The species flies from the beginning of May to the middle of June with a maximum in the third decade of May and the first decade of June (Vasic, 1954 cit. Camprag, 1973) and then from the end of July to the beginning of October. The maximum of the population is in the first two decades of August. According to Tanasievic, Ilic (1969) the flight of the first generation is May-June, and of the second-July-August. A mass flight of the first generation is in the third decade of May and the beginning of June. They pupate in mid-July, so the mass flight of the second generation is during August, which is consistent with our investigations (Kolektiv autora, 1983). According to Vukasovic et al., 1962, females lay eggs in May when there is an increased flight in our research, the butterflies of the second generation fly in August until the beginning of September.

In 1972, the flight dynamics of *A. segetum* is from April to October. The population of this species was much larger compared to 2020 - 2022 with a total of 2684 specimens caught with the light trap. The first peak of the appearance of butterflies is in May with 253 species, and the second in August with 689 butterflies caught (Todorovski, Vasilev, 1973). While in 1992-1993 and 1995 the species was represented from May to September with two peaks in June and August, with a total of 445 butterflies in 1992, 48 in 1993 and 72 individuals in 1995, and with a total of 183 individuals in 1994 with two peaks in June and September.

The species *A. ipsilon* is known as the cutworm, the so-called black ipislon owl. Forewings are grey/dark brown with characteristic dark brown spots and dark lines, often lighter in color towards the edge of the wing. The hindwings are whitish to gray with darker venation and a dark brown outer marginal stripe (Figure 4).

Figure 4. *A. ipsilon*

In 2020 a total of 14 adults were caught, 18 in 2022 and 25 in 2021. The *A. ipsilon* flight in 2020 was determined in June and from August to the first decade of September (Table 7).

Table 7. Quantitative representations of *A. ipsilon* in 2020 - method of light trap

Decade	Months							
	III	IV	V	VI	VII	VIII	IX	X
1	/	/	/	/	/	7	1	/
2	/	/	/	2	/	1	/	/
3	/	/	/	/	/	3	/	/
Total no /months	/	/	/	2	/	11	1	/
Total number		14						

Table 8. Quantitative representations of *A. ipsilon* in 2021 - method of light trap

Decade	Months							
	III	IV	V	VI	VII	VIII	IX	X
1	/	/	/	1	1	7	1	/
2	/	2	/	4	/	1	1	/
3	/	3	/	2	1	1	/	/
Total no /months	/	5	/	7	2	9	2	/
Total number		25						

The moth's flight in 2021 was determined in second and third decade of April, then in June to the first decade in July and from the end of July to the middle of September. The moths flight in 2022 was determined in second decade of June to the first decade of September. The maximum flight of moths was in the first decade of August 2020/21 and second decade of August 2022 (Table 8 and 9).

Table 9. Quantitative representations of *A. ipsilon* in 2022
- method of light trap

Decade	Months							
	III	IV	V	VI	VII	VIII	IX	X
1	/	/	/	/	1	3	1	/
2	/	/	/	2	1	4	/	/
3	/	/	/	4	1	1	/	/
Total no /months	/	/	/	6	3	8	1	/
Total number		18						

The caterpillars of the first generation do damage in the middle of May (Kolektiv autori, 1983), so in June is the flight of the adults as in our investigations 2020 and 2022.

In 1972, the flight dynamics of *A. ipsilon* is from March to October. The population of this species was much larger compared to 2020 - 2022 with a total of 1203 specimens caught with the light trap. The first peak of butterflies is in April with 172 specimens, the second in June with 448 butterflies, and the third in August with 310 butterflies caught (Todorovski, Vasilev, 1973). While in 1992 there was only a catch of 6 specimens, in 1993 4 specimens, and in 1995 14 butterflies. In 1994, the species was represented in June, then from August to November with a total of 48 butterflies, with the maximum number in August (Vasilev et al. 1996).

CONCLUSIONS

The studied species are active at night, which is why they are easily caught with a light trap.

In 2020 a total of 76 adults of H. armigera, 80 of A. segetum and 14 of A. ipsilon were caught, 74 moths of H. armigera, 45 of A. segetum and 25 adults of A. ipsilon in 2021, and 68 adults of H. armigera, 72 of A. segetum and 18 of A. ipsilon in 2022.

The small number of specimens caught in the light trap during 2020- 2022 indicates the successful timely control of these species.

For successful monitoring of these species we must be careful that these light traps are placed among the tobacco plantations away from the urbanized area because the increased number of lights in the surrounding facilities would directly reduce the catch in the trap.

For the control of Noctuidae species, it is required constant monitoring of the flight of the adults.

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TRANSFER AND BIOACCUMULATION OF Cd IN A SOIL-PLANT-APHIDS SYSTEM

Viktorija Bosheska[1], Biljana Jordanoska Shishkoska[2], Valentina Pelivanoska[2], Aleksandra Cvetkovska-Gjorgjievska[3]

[1] SUGS "Orce Nikolov", "Ilinden" Blvd. No. 101, 1000 Skopje, Republic of North Macedonia
[2] University "St. Kliment Ohridski"- Bitola, Scientific Tobacco Institute - Prilep, Republic of North Macedonia
[3] Institute of Biology, Faculty of Natural Science and Mathematics, Ss. Cyril and Methodius University in Skopje, Arhimedova 5, 1000 Skopje, Republic of North Macedonia
e-mail:Bosheskav@outlook.com

ABSTRACT

This study investigated the biological transfer and bioaccumulation of Cd from a contaminated substrate in tobacco (*Nicotiana tabacum* L.) and aphids (*Myzus persicae* Sulz.). The soil samples were treated with Cd solutions at concentrations of 0, 25, 50, 75, and 100 mg/l, and the experiment was conducted in a greenhouse. Tobacco plants were infested with aphids and samples were collected for microelement content analysis. The total concentration of Cd in the soil samples was determined using aqua regia, and the Cd content of tobacco plants and aphids was analyzed using atomic absorption spectrometry. Also, the bioaccumulation factor (BAF) was calculated. The Cd content in tobacco leaves increased with higher Cd concentrations in the soil, indicating successful uptake. The BAF values for the soil-tobacco system were >1, suggesting accumulation of Cd in tobacco plants. However, the BAF for the tobacco-aphid system was <1, indicating no biomagnification of Cd in the aphids.

Keywords: bioaccumulation, cadmium, trophic levels, *Nicotiana tabacum L.*, *myzus persicae* Sulz.,

ТРАНСФЕР И БИОАКУМУЛАЦИЈА НА Cd ВО СИСТЕМОТ ПОЧВА-РАСТЕНИЕ-ЛИСНИ ВОШКИ

Оваа студија го истражува биолошкиот трансфер и биоакумулацијата на Cd од контаминиран супстрат во тутун (*Nicotiana tabacum L.)* и лисни вошки (*Myzus persicae Sulz.*). Почвените примероци беа третирани со раствори на Cd во концентрации од 0, 25, 50, 75 и 100 mg/l, а експериментот се спроведе во стакленик. Тутунските растенија беа инфестирани со лисни вошки, а примероците беа собрани за анализа на содржината на микроелементот. Вкупната концентрација на Cd во почвените примероци беше определена со употреба на царска вода (*aqua regia*), а содржината на Cd во тутунските растенија и лисните вошки беше анализирана со атомска апсорпциона спектрометрија. Исто така беше пресметан и факторот на биоакумулација (BAF). Содржината на Cd во листовите од тутунот се зголеми со зголемување на концентрациите на Cd во почвата, што укажува на успешна апсорпција. Вредностите на BAF за системот почва-тутун беа >1, што сугерира акумулација на Cd во тутунските растенија. Сепак, BAF за системот тутун-лисни вошки беше <1, што укажува на отсуство на биомагнификација на Cd во лисните вошки.

Клучни зборови: биоакумулација, кадмиум, трофички нивоа, *Nicotiana tabacum L., Myzus persicae Sulz.*

INTRODUCTION

Agroecosystem contamination by cadmium (Cd) poses a significant challenge, as this metal can persist in soil for extended periods of time (Gall et al., 2015). The toxic effects of Cd can disrupt ecological interactions and negatively affect ecosystem structure and function (Dar et al., 2015). The food chain is a fundamental ecological concept that illustrates the flow of energy and nutrients at different trophic levels. Each stage in this chain is crucial for maintaining the ecological balance and ensuring ecosystem health. According to Tripathi et al. (2021), cadmium (Cd) poses significant ecological risks as it can biomagnify and bioaccumulate in organisms at higher trophic levels. As the foundation of numerous food chains, plants facilitate the transmission of metals to subsequent trophic levels, including herbivorous insects (Green et al., 2010; Dar et al., 2015). Tobacco (*Nicotiana tabacum* L.) serves as a significant model for research on the accumulation and phytoextraction of heavy metals from the soil (Angelova et al., 2004). The focus of this study was the aphid *Myzus persicae* Sulz., which is prevalent in all tobacco-growing regions of our country, and targets commercial varieties of *Nicotiana tabacum* L., as reported by Krsteska (2016). Aphids can bioaccumulate and selectively remove an average of 50-60 % of chemical substances, including potentially toxic metals (Kamiński et al., 2021). Therefore, the following question arises: Does a higher concentration of Cd at a particular trophic level result in a correspondingly increased accumulation at subsequent trophic levels of the food chain, and is it possible for Cd to be retained at elevated concentrations at any of the trophic levels? The primary objective of this study was to investigate the biological transfer and bioaccumulation of Cd from a contaminated substrate to *Nicotiana tabacum* and aphids (*Myzus persicae* Sulz.) as primary consumers. This study aimed to monitor changes in the concentrations of heavy metals during their transport through the soil-tobacco-aphid trophic chain resulting from treatment with different concentrations of cadmium.

MATERIAL AND METHODS

The soil samples used in this study were obtained from uncontaminated soil in the experimental fields at the Tobacco Scientific Institute in Prilep (TSIP). These samples were air-dried and placed in pots, which were then treated with Cd solutions at concentrations of 0, 25, 50, 75, and 100 mg/l, made from standard reference solutions (Merck, 1000 mg/l). A total of 20 pots were utilized, with four replicates for each concentration level, and samples were collected from these pots to analyze the soil microelement content and soil parameters. The experiment was conducted in a greenhouse between June and September 2022. Twenty healthy plants obtained from TSIP nursery beds were transplanted. The plants were regularly watered, and during the vigorous growth phase of tobacco, just before flowering, they were infested with aphids obtained from the natural populations. The aphids were reared in a cage on one of the control groups and new generations of aphids were collected and transferred to tobacco samples using an exhaustor. Three weeks after the infestation, aphid samples were collected from each pot. Some aphids were analyzed for microelement content, whereas others were frozen to serve as food for ladybugs (*Coccinella septempunctata*, Coleoptera: Coccinellidae). The feeding experiment for colonizing ladybugs was set up in petri dishes, but was incomplete, therefore the research concluded with the feeding of the aphid samples.

Analysis of soil samples for pH, total nitrogen, humus, easily available phosphorus, and potassium

The soil samples were analyzed in compliance with ISO 11464:2006 criteria. The samples were air-dried, ground, and sieved through a mesh with a 2 mm opening. The assessed parameters included pH, total nitrogen, humus, easily available phosphorus, and potassium (Pelivanoska, 2012). The available forms were extracted according to ISO 14870 standards.

Analysis of Cd

The total concentration of metals in the soil samples was determined using aqua regia as described by Pelivanoska (2012). Additionally, the Cd content of tobacco plants was determined. The plants were washed to remove contaminants and then rinsed with distilled water. Subsequently, the samples were placed in paper bags, dried at 75°C for 12 h, and ground into powder. The plant material and aphid samples were dissolved in a CEM microwave oven following the method outlined in US EPA Method 3050 B (2007). The microelement content was determined by atomic absorption spectrometry using a Varian 220 A instrument.

Analysis of bioaccumulation factor

The bioaccumulation factor (BAF) was calculated as the ratio between the metal concentration in the plant and that in the soil, as well as the ratio of the metal concentration at the second trophic level (aphids) to that at the first trophic level (tobacco) (Butt et al., 2018).

RESULTS AND DISCUSSION

The results of the soil parameter analysis are presented in Table 1. The results indicated that the pH of the samples decreased as the concentration of the cadmium solution increased to 75 mg/l, whereas a slight increase was observed at 100 mg/l. The addition of Cd did not appear to significantly affect the humus content in the soil, although minor variations were evident. It is important to note that low soil pH can lead to increased bioavailability of heavy metals for absorption by tobacco plants, as reported by Golia et al. (2009).

Table 1. Soil Parameters of the Examined Soil Samples Under Different Treatments

Treatment level, mg/l	pH (H_2O)	pH (KCl)	Humus, %
Control group	7.42	6.69	1.96
25	7.42	6.61	2.03
50	7.34	6.54	2.15
75	7.16	6.36	2.07
100	7.18	6.4	1.95

Soil Cd content

Table 2 presents the Cd levels in the soil before planting. The results indicate that the concentration of cadmium (Cd) increases in a proportional manner with the increasing concentrations of the applied standard solution, ranging from 4.1 mg/kg at the 25 mg/kg treatment to 24.7 mg/kg at the 100 mg/kg treatment.

Table 2. Cadmium Content of the Examined Soil Samples Under Different Treatments

Treatment level, mg/l	Cd (mg/kg)
Control group	<DL
25	4.1
50	10.5
75	21.0
100	24.7

Plant uptake of Cd

Table 3 presents the Cd content in the examined tobacco leaves, where an increase in cadmium (Cd) content was observed compared to the initially added solutions in the soil. The concentration of cadmium (Cd) was insignificant in the control group; however, it demonstrated a progressive increase with higher treatment levels, reaching a substantial peak at 100 mg/l. This poses a risk of bioaccumulation in the food chain and is a potential hazard for plants.

Table 3. Cadmium Content in the Examined Tobacco Leave

Treatment level, mg/l	Cd (mg/kg)
control group	0.1
25	7.8
50	17.5
75	18.8
100	33.4

Cd accumulation in aphids

Table 4 displays the Cd levels in the aphid samples. The increase in Cd content in the aphids associated with the increase in treatment concentrations indicated a minimal transfer of Cd from the soil to the aphids via the plants.

Table 4. Microelement Content in the Examined Aphid Samples

Treatment level, mg/l	Cd (mg/kg)
Control group	<DL
25	0.3
50	0.7
75	1.3
100	2.9

Based on the data obtained for Cadmium (Cd), it is possible to assess potential biomagnification by observing the increase in the Bioaccumulation Factor (BAF) from soil to tobacco and from tobacco to aphids (Figure 1). The Bioaccumulation Factor (BAF) for Cd increased sharply at 25 mg/l and then gradually decreased, indicating a potential initial tolerance, accumulation saturation, or inhibition mechanism at higher concentrations. Considerable bioaccumulation factor values, particularly at low contamination levels, indicate that tobacco plants can readily accumulate Cd from the soil. BAF values for Cd uptake from tobacco to aphids were noticeably low, with all values falling below 0.1. This suggests that a minimal amount of Cd was transferred from tobacco to aphids. Nevertheless, despite these low values, the BAF values indicate a certain level of Cd transfer from plants to aphids.

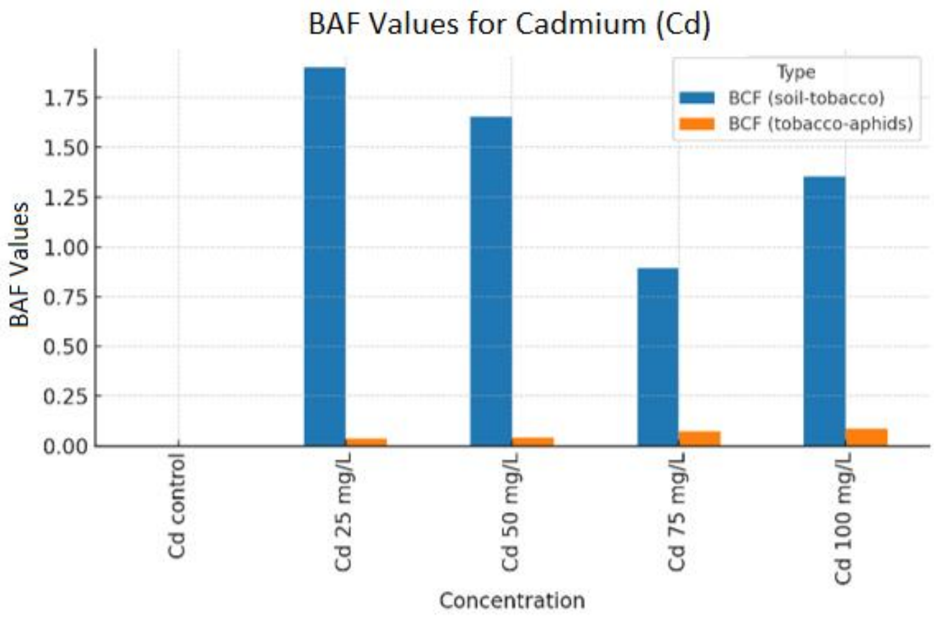

Figure 1. Bioaccumulation Factor (BAF) from Soil to Tobacco and from Tobacco to Aphids

Following the analysis of cadmium (Cd) bioaccumulation in *Myzus persicae*, this discussion aims to contextualise our findings within the broader field of ecological studies on heavy metal dynamics. The findings revealed low bioconcentration factor (BCF) values, all below 0.1, suggesting a minimal transfer of Cd from tobacco to aphids. Our findings of low BCF values in *Myzus persicae* are consistent with previous research that reported limited bioaccumulation of cadmium in aphid-plant systems. For instance, Green et al., (2006) observed no significant biomagnification of Cd in *Sitobion avenae* fed on barley (*Hordeum vulgare*), which they attributed to the restricted translocation of Cd within the plant. Similarly, Green et al., (2010) demonstrated that the Cd transfer coefficients from wheat to Sitobion avenae aphids were below 1 at all treatment concentrations. Butt et al., (2018) also found that Cd did not accumulate significantly in Sitobion avenae from Trifolium alexandrinum, with an average bioaccumulation factor below 1. While our findings align with those noting minimal bioaccumulation, it is crucial to consider studies that have observed higher Cd bioaccumulation rates in different aphid-plant systems. Dar et al., (2015) reported high Cd concentrations in *Lipaphis erysimi*, with transfer coefficients ranging from 0.8-1.7. Similarly, Green et al., (2003) found transfer coefficients ranging from 0.85 to 1.6 for *Sitobion avenae* feeding on *Triticum aestivum*. These contrasting findings indicate that Cd transfer and accumulation can vary significantly among different aphid and plant species, suggesting that environmental Cd levels, and plant and aphid physiology play a role in these variations. Our study contributes to this broader understanding by highlighting the variability of Cd bioaccumulation across different ecological contexts.

The mechanisms underlying Cd transfer in aphid-plant systems involve complex interactions between plant resistance and aphid adaptation strategies. One key factor is the regulation of Cd concentration in the plant phloem, which determines the amount of Cd available for aphids to ingest. In addition, aphids possess regulatory mechanisms to manage Cd accumulation, ensuring that metal concentrations do not reach toxic levels. According to Naikoo et al. (2021) and Dar et al. (2015, 2017), aphids can partially regulate Cd bioaccumulation through excretion of this metal in honeydew; However, Cd content in honeydew does not increase proportionally with Cd concentration in aphids, implying that excretion through honeydew is not the sole regulatory mechanism. This regulation may vary among aphid species, as suggested by Crawford et al., (1995), thereby contributing to the differences observed across studies. The levels of Cd in aphids were considerably lower than those in tobacco plants, which may be attributed to the efficiency of the regulatory mechanisms in *Myzus persicae*. Further research on the regulatory pathways of *Myzus persicae* is crucial to gain a better understanding of Cd transfer and accumulation, and the minimal transfer of Cd from tobacco to *Myzus persicae* suggests a limited risk of biomagnification in this specific food chain. These findings contribute to our understanding of heavy metal dynamics, emphasising the importance of system-specific studies to assess the ecological impact of metal pollution.

CONCLUSIONS

The bioaccumulation and trophic transfer of Cd in the food chain highlights the complex dynamics of heavy metal movement within ecosystems. These interpretations underscore the need for further research to understand the mechanisms of bioaccumulation and trophic transfer of heavy metals as well as their potential consequences on ecosystems. Understanding these dynamics is crucial for developing strategies to manage the risks associated with heavy metals in agroecosystems. The implementation of regulatory measures and agricultural practices aimed at reducing heavy metal inputs into the soil, along with regular monitoring of metal concentrations in agricultural crops and associated organisms, play a vital role in ensuring food safety and environmental health. These findings emphasize the importance of ecological studies to understand the natural cycles of pollutants and their effects on different species. Further research is required to identify the specific genetic and molecular mechanisms involved in the regulation of heavy-metal accumulation in plants and insects.

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Original Scientific Paper

INFLUENCE OF ELEVATED DELTAMETHRIN APPLICATION RATES ON MICROELEMENT UPTAKE BY ORIENTAL TOBACCO

Biljana Jordanoska Shishkoska, Bojana Dimovska Gonovska, Marjana Glusheska, Vesna Krsteska
Scientific Tobacco Institute, St. Kliment Ohridski University, Kičevska bb, 7500 Prilep, Republic of Macedonia;
e-mail: biljana.jordanoska@uklo.edu.mk

ABSTRACT

Crop pest protection strategies and practices in agriculture are still heavily reliant on chemical pesticides. Pyrethroid pesticides are insecticides of intense interest in tobacco protection practices because of their desirable environmental properties: short persistence and nontoxicity to mammals. Although recommendation for their usage is strictly declared by the manufacturer, there are always deviations. Field experiment was caried out in order to investigate the effects of different deltamethrin application rates and their impact on the content of two selected microelements (Cu and Zn) in oriental tobacco plants. The study provides an insight into accumulation of these microelements in the root and the shoot system of the tobacco plant.

Keywords: deltamethrin, oriental tobacco, copper, zinc

ВЛИЈАНИЕТО НА ЗГОЛЕМЕНИ ДОЗИ НА ДЕЛТАМЕТРИН ВРЗ СОДРЖИНАТА НА МИКРОЕЛЕМЕНТИ ВО ОРИЕНТАЛНИОТ ТУТУН

Стратегиите и практиките за заштита на насадите во земјоделството сѐ уште се во голема мера зависни од хемиските пестициди. Пиретроидните пестициди каков што е делтаметринот се инсектициди од голем интерес за употреба во заштитата на тутунот. Ова се должи на нивните пожелни еколошки својства: краток период на распаѓање и нетоксичност за цицачите. Иако препораката за нивна употреба е строго декларирана од производителот, секогаш има отстапувања. Направен е полски опит со цел да се истражат ефектите од примена на различни дози од делтаметрин и нивното влијание врз содржината на два избрани микроелементи (Cu и Zn) во растенијата на ориенталски тутун. Студијата дава увид во акумулацијата на овие микроелементи во коренот и надземниот дел на растението тутун.

Клучни зборови: делтаметрин, ориентален тутун, бакар, цинк

INTRODUCTION

Agriculture is a fundamental source of our food supply with the ideal objective to provide safe products with minimal adverse environmental impacts. Optimisation of the crop production is essential and use of agrochemicals including synthetic pesticides is inevitable. A variety of pesticides belonging to different chemicals groups, organophosphates, carbamates and anthranilic diamides, spinozines, pymetrozines, oxadiazines, synthetic pyrethroids are recommended for use. Some of these pesticides are broad-spectrum and can control more than one target. At the same time, some products containing the mixtures of two or more pesticides, generally organic phosphate plus synthetic pyrethroid (OP+SP), are available.

Deltamethin is one of the most potent insecticides (Elliot, 1977) and appears very promising for controlling insect pests found to infest various vegetable and field crops. In recent years it's degradation rate and pathways in plants have been reported. Ruzo and Caslda (Ruzo & Castda, 1979) and Cole et al. (Cole et al. 1982) characterized a large number of extractable metabolites following application of the insecticide upon plant foliage. The information on the metabolic fate of deltamethrin in crops is important for an improved understanding of its practical uses in agriculture. Pyrethroid pesticides are also substances of intense interest for use in tobacco protection because of their desirable environmental properties of short persistence and nontoxicity to mammals (Baker et al. 1982). Their features, combined with a broad spectrum of pesticidal activity made them alternatives to the older organochlorine compounds and the natural pyrethrins.

Although recommendation for the usage of the pesticides is strictly declared by the manufacturer, there are always deviations. Their excessive and uncontrolled application causes environmental damage (Shahid & Saghir Khan, 2022). They affect most of the soil microorganisms, subsequently disturbing soil nutrition properties and cause a substantive reduction in agricultural production.

Oriental tobacco production in our country holds a valuable economic impact. According to Campbell (2000), basic micronutrients for the development of tobacco culture are: Fe, Mn, Zn, Cu and B. Copper participates in numerous physiological processes in plant cells and is a cofactor of many proteins. Zinc is an essential component of proteins in plants and has a direct or indirect influence on photosynthesis, health, chlorophyll biosynthesis, and carbohydrate metabolism (Foy et al., 1978, Bowen, 1979). Starting from the importance of microelements for the tobacco plant, one of the aims of this study was to investigate does different Deltamethrin application rates have influence on the copper and zinc content in oriental tobacco and the soil where it is cultivated. Furthermore, copper and zinc accumulation rate were observed in different oriental tobacco plant organs.

MATERIAL AND METHODS

The field experiment was caried out during 2022. The plot was set with untreated control and different Deltamethrin rates in three replicates. The treatments included recommended dose of Delthametrin (25 g/L, Polux) and 30%, 50%, 70%, 100% increased dose. Tobacco samples (root, stem, leaf, blossom) and soil samples were collected in September 2022. Plant samples were washed carefully to remove any adhering soil particles and rinsed with redistilled water. The plant material was dried and homogenized to a constant weight after drying at 75^{o}C for 12 hours. For the analysis, plant samples (0.5 g) were put in Teflon vessels with 5 ml concentrated HNO_3 (trace pure, Merck, Germany) and 2 ml H_2O_2 (30%, m V-1, Merck, Germany) the vessels were closed, tightened and placed in the rotor of a microwave digestion system (Milestone, Ethos Touch Control). The plant samples were digested at 180^{o}C. After digestion and cooled samples were quantitatively transferred into 25 ml calibrated flasks (Bačeva et al., 2012).

Pretreatment of soil samples for physico-chemical analysis was done in accordance to ISO 11464 (2006). Soil samples were collected before planting and after harvest from both locations and analysed for soil texture, pH, total nitrogen content, organic matter content, carbon content, available phosphorus and available potassium content and Cu and Zn (Pelivanoska, 2011). The obtained solutions of tobacco and soil samples were analysed with the flame atomic absorption spectrometry (FAAS), Varian 220A.

RESULTS AND DISCUSSION

All soil properties including the Cu and Zn content of soil samples are presented in Table 1. According to the obtained data, soil sampled from the experimental field has low humus content, poorly acidic soil pH and medium content of available phosphorus and potassium. Soil samples were analyzed before and after harvesting and the end of the vegetation. The results are given for every studied variant, treated with different Deltamethrin doses.

Zn content in soil depends on the geochemical nature, organic matter content, soil texture and pH. Zn content in soil is from 10 to 300 mg/kg (Kabata-Pendias, 2011). Plants absorb zinc from the soil solution through the roots mainly in the form of Zn^{2+} and organic chelates (Golia et al., 2002). In our samples, Zn content is near the average value of 55 mg/kg found in arable land form Pelagona area (Jordanoska et al. 2012, 2018).

Copper in agricultural soils is usually found in the range of 1 to 50 mg/kg (Hodges, 1995). Plants absorb copper from the soil solution through the roots in the form of organic complexes or Cu^{2+}. Jordanoska et al. 2018 found Cu in tobacco soils form Pelagonia area in the range from 5 to 39 mg/kg. Based on the results from the analyzed soil samples with different deltamethrine treatments, (Table 1) no regularity was observed in Cu and Zn content in the samples where tobacco plants were treated with increased concentrations.

Table 1. Basic soil properties, Cu and Zn content of soil samples with different Deltamethrin dosage rates

Soil samples analyses	OM %	TN %	pH H_2O	pH KCl	Available P mg/100 g	Available K mg/100 g	Cu mg/kg	Zn mg/kg
untreated control	1.18	0.058	6.91	5.88	20.59	20.61	23.15	49.25
untreated control after harvest	1.07		7.05	6.19	24.06	18.03	23.45	50.12
recommended dosage	1.09	0.057	6.81	5.92	25.15	19.85	24.58	51.24
recommended dosage +30 %	1.08	0.056	6.82	5.98	25.12	19.75	23.85	49.56
recommended dosage +50 %	1.07	0.058	6.82	5.96	25.10	19.82	23.98	50.18
recommended dosage + 70 %	1.08	0.056	6.83	5.97	25.18	19.78	24.12	51.02
recommended dosage +100 %	1.06	0.056	6.82	5.96	25.12	19.85	24.15	49.89

The content of Zn in the tobacco plant organs are given in Table 2. The main source of zinc for plants is from the soil. The natural content of Zn in tobacco ranges from 30-79 mg/kg (Grabuloski and Simonoska 1985). In their research, the average values obtained for the oriental tobacco type are close to 43.50 mg/kg. According to the data of Golia et al. (2009) in oriental tobacco varieties zinc content ranges 5.4 -50 mg/kg. Jordanoska et al. 2018 fount 8-52 mg/kg Zn, with average values of 20 mg/kg is samples from Pelagonia region. Some authors consider zinc to be very mobile in plants, while others point to its moderate mobility, which is conditioned by the age of the plants. In young plants, 75% of the total absorbed Zn passes into the above-ground organs, while in older plants, only 20-30% is found in the above-ground organs (Baumeister and Erst, 1978). The obtained values for the content of zinc in tobacco leaves are in agreement with data indicated in other literary sources (Grabuloski and Simonoska, 1985; Tso, 1989, Pelivanoska 2007, Golia et al., 2009, Jordanoska et al., 2018). As it can be seen in the Table 2 the highest content of Zn in the roots of the tobacco plants is determined in the samples from the untreated control. Compared to the control all root samples from different treatments have lower content of Zn. Difference in the accumulation of the Zn is observed in the organs of the tobacco plant from all tested treatments, with highest obtained values in the sampled flowers. The most likely reason for the is that the pre-flowering and flowering period is a period when there is significant plant consumption of Zn, Fe and Cu.

As whole plant, given as total in the Table 2, we can see that control has lowest content of Zn and samples treated with recommended dosage have highest content of the observed microelement.

Table 2. Zn content of plant material with different Deltamethrin dosage rates

Zn, mg/kg	Root	Stem	Leaves	Flowers	Total
Control	14.43	4.65	8.94	15.32	28.91
recommended dosage	7.78	9.44	11.62	18.55	47.38
recommended dosage +30 %	5.89	5.11	9.52	23.94	44.44
recommended dosage +50 %	5.50	3.40	8.82	14.66	32.38
recommended dosage + 70 %	7.63	4.34	10.38	23.24	45.58
recommended dosage +100 %	9.58	4.67	8.70	16.83	39.77

The distribution of Cu content in different oriental tobacco organs is presented in Table 3. Similar as Zn, roots sampled from the control treatment have highest content of Cu. Cu content in tobacco leaves of 5 to 15 mg/kg is found in most of the studies (Adamu et al., 1989; Bell et al., 1992 и Bergmann, 1992, Jordanoska et al., 2018). According to Collins et al. (1961), in Virginia tobacco, the copper content is in the interval of 14.9 and 21.1 mg/kg, while in the researches of Zaprjanova & Bozhinova (2004) it is 16.9-25.8 mg/kg. According to the data of Golia et al., (2009) mean Cu concentrations of 66 mg/kg were determined in Berley tobacco, 37 mg/kg in Virginia tobacco and 23 mg/kg in Oriental tobacco. In our study Cu content of the tobacco leaves is much lower that all mentioned authors but in the range as normal content of copper in plants is from 5 to 30 mg/kg (Kabata-Pendias, 2011).

Table 3. Cu content of plant material with different Deltametrin dosage rates

Cu, mg/kg	Root	Stem	Leaves	Flowers	Total
Control	2.50	0.96	0.94	1.84	6.24
recommended dosage	1.27	1.67	1.26	2.37	6.57
recommended dosage +30 %	0.84	0.62	0.45	2.73	4.64
recommended dosage +50 %	0.92	0.72	0.74	2.20	4.57
recommended dosage + 70 %	1.13	0.97	0.73	4.05	6.88
recommended dosage +100 %	1.42	1.75	0.74	2.76	6.66

The highest contents of Cu in plant as total is determined from the treatment with 70 % increasement from the recommended dosage.

The translocation of copper between different plant parts plays an important role in its utilization. It has been observed that in conditions of its deficiency, or in conditions of excessive concentrations, the root has a strong capacity to retain it (Mrvić et al., 2009). In the root, copper binds to the cell walls and remains immobile and only a small part reaches the young organs and therefore they are most often exposed to a lack of this element. (Mrvić et al., 2009). In order to present bioaccumulation of both studied elements in the oriental tobacco plant organs we use the biological transfer factor (BTF). BTF is calculated as the ratio of the content of the examined element in the above-ground tissues of the plant and the root. This factor indicates the efficiency of the transfer of elements in the aerial parts of the plant. The calculated values are presented on Figure 1.

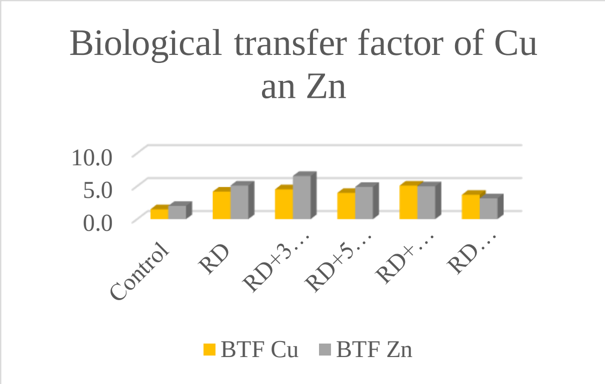

Figure 1. The biological transfer factor (BTF) as a measure of the efficiency of the transfer of elements in the aerial parts of the plant

As we can see in Figure 1, Zn has higher accumulation factor than Cu in all treatments from the preformed field experiment, except in the treatment with increased rate of 70%. This means that oriental tobacco has a higher tendency to transfer and accumulate Zn rather than Cu in the aerial organs.

CONCLUSIONS

The aim of our investigation was to determine the whether elevated deltamethrin application rates have impact on the Cu and Zn content of soil samples and the oriental tobacco plant. Compared to the control, higher content of Zn is determined in the oriental tobacco plants that are treated with deltamethrin, even with the recommended dosage. It was established that oriental tobacco has a higher tendency to transfer and accumulate Zn compared to Cu. Higher tendency of transfer and accumulation of Cu prevails in the treatment with increased rate of 70% from the recommendation dose. Deltamethrin dosage had no influence on soil samples. This study is only one approach that provides input in the further monitoring that will prevent the negative impact of pesticides on soil, tobacco and tobacco products. The presented study is helpful for recommendations for commercial dosage use that is environmentally responsible. According to our findings, content of the Cu and Zn are consistent with the data found in the referent sources.

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TECHNOLOGY OF TOBACCO SEEDLING PRODUCTION AND COSTS FOR THE PRODUCTION OF 20 SEEDBEDS OF TOBACCO SEEDLINGS

Marjana Glusheska[1], Biljana Jordanoska Shishkoska[1], Vesna Krsteska[1], Marjan Trajkoski[2], Kristina Hristovska[1]

[1] *"St. Kliment Ohridski" University - Bitola, Scientific Tobacco Institute - Prilep, str."Kichevska" bb. Prilep, Republic of North Macedonia*

[2] *Tobacco Company - Prilep, str."Prilepski braniteli"85, Prilep, Republic of North Macedonia*

e-mail: marijana.gluseska@uklo.edu.mk

ABSTRACT

Tobacco production technology includes two stages: seedling production and field production. To plant an area of 1 ha with tobacco it is necessary to sow 20 seedbeds. The seedbeds were cold-prepared, standard (length 10m and width 1m). Tobacco seeds of Prilep 66 9 variety were sown at a seeding rate of 4.5g/10m^2. For each work operation during seedling production, the required time and the number of workers were measured and then the cost and labor were calculated. The cost price for hiring workers for the production year 2022 was € 2.9 per hour. The cost price for producing 20 seedbeds of tobacco seedlings (required for an area of 1 ha) amounts to a total of € 1085.

Key words: tobacco, seedling production, costs, price.

ТЕХНОЛОГИЈА НА ПРОИЗВОДСТВО НА ТУТУНСКИ РАСАД И ТРОШОЦИ ЗА ПРОИЗВОДСТВО НА 20 ЛЕИ ТУТУНСКИ РАСАД

Технологијата на производство на тутун опфаќа две фази и тоа: расадопроизводство и нивско производство. За да се расади површина од 1 ха со тутун потребно е да се посеат 20 леи. Леите беа ладно усовршени, стандардни (должина 10 m и ширина 1 m) и беше користена норма на сеидба од 4,5g/10m^2 тутунско семе од типот прилеп 66 9. За секоја работна операција при расадопроизводството беше мерено потребното време и бројот на работници кои беа вклучени за извршување на истите, а потоа беше вкалкулиран и трошокот и работната сила. Цената на чинење за ангажирање на работници за производната 2022 година беше € 2,90 од час.

Клучни зборови: тутун, расадопроизводство, трошоци, цена.

INTRODUCTION

Tobacco industry is one of the most organized agricultural industries in our country. Over the past five years, tobacco production in the Republic of North Macedonia has ranged from 20 000 to 25 000 tons and is one of the largest in the Balkans. In the past, more than 30 000 families relied on tobacco production for their livelihoods, but in recent years, there has been a decline to around 20 000 families.

The production technology of tobacco itself is specific, starting from the production of tobacco seedlings, cultivating tobacco in fields and culminating in post-harvest processing, including tobacco curing and home handling. From an economic standpoint, tobacco plants are cultivated for their leaves, and thus the proper use of agrochemical operations is of enormous importance and directly impacts the realized income.

Soil represents the medium for growing and developing of tobacco plants, so it is particularly important to understand it for achieving both qualitative and quantitative tobacco production. Optimized production entails the rational utilization of mechanization, labor and working hours for the necessary operations.

Seedling production is an inevitable stage in tobacco production and producing quality seedlings is a crucial precondition for successful tobacco production in the fields. Besides the quality of the seedlings, it is also of paramount importance to produce a sufficient quantity of tobacco seedlings for the planned area. Also, the seedlings should be ready for transplanting within the optimal timeframe for the production region.

Developing an effective technology for tobacco seedling production requires a good understanding of the biology of the seedling, which goes through multiple developmental phases. Application of high-quality and timely agronomic measures is also very important.

MATERIAL AND METHODS

For the production of tobacco seedlings, the following types of seedbeds exist (Uzunoski, 1985):

- Ordinary or cold seedbeds
- Warm seedbeds

Cold seedbeds are further divided into raised and sunken beds. Raised beds are those lying above the path level, while sunken beds are those lying below the path level. Warm seedbeds are classified as warm and semi-warm beds.

For the purposes of this research, the seedbeds were cold prepared i.e. cold seedbeds covered with polyethylene fabric. The work process included several phases, during which the time and number of workers were measured:

- Autumn and spring soil preparation for seedling production
- Preparation of the seedbeds
- Sowing the seedbeds
- Herbicide treatment
- Regulation of the air-thermal regime
- Seedbeds fertilization
- Irrigation
- Protection of tobacco seedlings
- Seedlings pulling

RESULTS AND DISCUSSION

To plant an area of 1 ha with tobacco, 20 cold prepared seedbeds were sown in the experimental field at the Scientific Tobacco Institute - Prilep. According to (Korubin-Aleksoska, 2004), recommended seeding rate for Prilep tobacco type, ranges from 3-5g/10m^2. In our case, the seeding rate was 4.5g/10m^2. Seed of the tobacco type Prilep, variety 66 9 was used. Seebeds were standard, measuring 10 meters in length by 1 meters in width.

Fig. 1 Tobacco seedbed with seedlings

Appropriate cultural practices were applied before sowing the seedbeds. Preparation of the soil consisted of one fall and two spring plowings. Fall plowing is an important cultural practice as it improves soil structure, enhances the soil's water-air regime and also improves soil fertility. It also destroys weed vegetation and prevents the overwintering of numerous parasitic diseases and pests (Mickovski, 2004). The formation of the beds was carried out using a seedbed shaper, followed immediately by manual bed correction (leveling, raking, soil compaction, edge correction, frame placement and covering with polyethylene fabric). This bed formation process required 8 hours and 4 workers (Table 1). After seeding, the tobacco beds were covered with burnt manure, and then herbicide treatment with Clomazone active ingredient was applied at a concentration of 1ml/10m^2. Burnt manure cover is used to provide sufficient warmth for the tobacco seeds during germination. During the 56-day seedling production period, timely and controlled irrigation was carried out. According to (Uzunovski, 1985), irrigation of the nursery is a very important agronomic measure. Initially, the tobacco beds were irrigated daily to provide enough moisture for seed germination. Additionally, timely weeding, regulation of the air regime of the beds (covering and uncovering the beds) were performed to obtain healthy and high-quality seedlings. According to the recommendation of experts (Scientific Tobacco Institute - Prilep, 2022) three treatments were carried out during seedling production to protect the tobacco seedlings from disease and pests. The first treatment was conducted on April 18, 2022, in the 4-leaf stage, applying a combination of two fungicides with Boscalid and Pyraclostrobin active ingredients at a concentration of 1.5g/10m^2 and Ametoctradin at 1ml/10m^2 to prevent the occurrence of black root rot in tobacco seedlings (Thielaviopsis basicola). According to (Tashkoski, 2021), black root rot is a widespread disease attacking the root system of the plant and can cause damage to both seedlings and tobacco planted in the field.

The second protection of the tobacco from diseases was carried out on May 3, 2022, with a fungicide containing Azoxystrobin active ingredient at a concentration of 1.5ml/10m^2. The third protection of the tobacco seedlings was performed with a combination of fungicides containing Boscalid and Pyraclostrobin at 1.5g/10m^2 + Ametoctradin at 1ml/10m^2 and an insecticide based on Deltamethrin at 0.5ml/10m^2. The entire protection process was carried out by 1 worker and required 4 hours (Table 1).

According to (Hawks, 1970), tobacco seedlings grow faster when fertilized with nitrate nitrogen. In our case, we had three fertilizations of tobacco seedlings with NH4NO3 34.5% at a concentration of 80g/10m^2. The fertilization was carried out by 2 workers for a total of 10 hours (Table 1). According to (Mickovski, 2004), if weeds are not removed from the tobacco beds, they will cover the seedlings, depriving them of light and nutritional space. Tobacco beds were kept free of weed vegetation to provide a clean environment and allow the tobacco seedlings to grow and develop unhindered. Weeding required 4 workers and 16 hours (Table 1) for the operation.

For the required area of 1 hectare, around 175 000 healthy seedling plants were used, i.e. 70 jute sheets with 2500 healthy and high-quality tobacco seedlings each. The required working hours for the pulling operation were 16, with 5 workers involved (Table 1).

Table 1. Number of workers and working hours per work operation

Work operation	Working hours	Number of workers
Preparation of 20 seedbeds	8	4
Sowing	5	2
Fertilizing	10	2
Regulation of the air-thermal regime	30	2
Irrigation	15	2
Protection of tobacco seedbeds	4	1
Weeding	16	4
Pulling	16	5

The cost of one working hour for hiring seasonal workers in our case was € 2,90. From Table 2, the total labor cost required for 20 seedbeds can be seen

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Table 2. Total cost for seasonal workers

Work operation	Working hours	Price per hour	Cost in EUR
Seedbeds preparation	32	2,90	93
Sowing	10	2,90	29
Fertilizing	20	2,90	58
Regulation of the air-thermal regime	60	2,90	174
Irrigation	30	2,90	87
Protection of tobacco seedbeds	4	2,90	12
Weeding	64	2,90	185
Pulling	80	2,90	232
Total	300		870

For nursery production of 20 tobacco seedbeds, 300 working hours were needed.
The cost for the workers was € 870 (Table 2).

From table 3 it can be seen that the repromaterials costs for 20 seedbeds amount to € 215.

Table 3. Total costs for repromaterials

Repromaterial	Cost in EUR
Irrigation water	13
Ammonium nitrate 34,5 %	5
Herbicide, insecticide and fungicide	12
Polyethylene fabric	89
Transport fuel	72
Burnt manure	24
Total	215

CONCLUSIONS

Based on the previously presented material, the following conclusions can be made:

- For planting an area of 1 ha, it is necessary to sow 20 cold-prepared seedbeds, which are enough to obtain healthy, high-quality and timely matured tobacco seedlings
- The seed production period lasted 56 days
- The price of renting seasonal workers for 1 hour is € 2,90
- The required number of working hours for nursery production is 300
- Total cost for seasonal workers amounts to € 870
- The cost of repromaterials is € 215
- The total cost for 20 seedbeds is € 1085 or for one seedbed is € 54,25

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