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Crude protein content in nine alfalfa cultivars and its relationship with dry matter yield

Daniela Kertikova*, Anna Ilieva, Todor Kertikov

Institute of Forage Crops, 5800 Pleven, Bulgaria

* -mail: d_kertikova@abv.bg

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SUMMARY

2006-2012 . -
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19 -
(21,91%), -
(19,99%).
- (24,86%-32,41%),
(12,64%-16,62%).
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During the period 2006-2012 nine alfalfa cultivars – Europa, Obnova 10, Pleven 6, Dara, Dama, Prista 2, Prista 3, Prista 4 and Mnogolistna 1 were studied. The aim of the research was evaluation of alfalfa cultivars on economic traits and selection in condition of competition. This article presents data on crude protein content in dry matter for five years of first cut, second cut including leaves and stems and on average, of all cuts.

Under the test conditions, an average of 19 cuts with the highest crude protein content is cv. Mnogolistna 1 (21,91%), and the lowest cv. Prista 2 (19,99%). By years the protein content in the leaves varies widely (24,86%-32,41%) than in the stems (12,64%-16,62%).

Over the years, different cultivars (Dama, Mnogolistna 1, Plevan 6, Prista 3 and Obnova 10) occupy the first position in the protein content of the stems, whereas in the leaves cv. Mnogolistna 1 has the highest content. The dry matter yield is in negative correlation with the crude protein

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(*Medicago sativa* L. ssp. *sativa*) (Bouton, 2012).

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(Krachunov et al., 2006).

(Monirifar and Abdollahi, 2014).

(Kertikova and Ilieva, 2000; Tucak et al., 2008; Annicchiarico et al., 2010; Bo Han et al., 2013; Kertikova, 2014).

(Rotili et al., 2001; Kertikova et al., 2000; Kertikova, 2008; Monirifar, 2011; Vasileva, 2012; Vasileva and Athar, 2012; Hu An et al., 2017).

- content. The alfalfa cultivars Pleven 6, Dara and Prista 3 have a good balance between yield and crude protein content in the dry matter.

Key words: alfalfa, cultivars, crude protein, cut, yield, correlation

INTRODUCTION

Cultivated lucerne (*Medicago sativa* L. subsp. *sativa*) is the most common forage species in the world (Bouton, 2012). The leading role of alfalfa in forage production is driven by its large productive capabilities. Alfalfa is one of the most economically important forage crops for our country. Many other advantages (multiple cuts, high yield, drought resistance, improvement of soil fertility, high protein content) make alfalfa a crop preferred by the farmers for growing. Alfalfa in a fresh stage or preserved as hay, haylage or dehydrate can be fed to all animals (Krachunov et al., 2006).

Variability for agronomic and morphological traits of alfalfa is frequently used in breeding programs for developing cultivars with a high forage production and better quality (Monirifar and Abdollahi, 2014). The alfalfa cultivars differ in productive potential, forage quality, drought resistance, both in optimal and limiting conditions of the environment (Kertikova and Ilieva, 2000; Tucak et al., 2008; Annicchiarico et al., 2010; Bo Han et al., 2013; Kertikova, 2014). Although alfalfa is commonly accepted as high protein feed, the increase in protein is always in the focus of researchers, both from an agro technical point of view and from a selection (Rotili et al., 2001; Kertikova et al., 2000; Kertikova, 2008; Monirifar, 2011; Vasileva, 2012; Vasileva and Athar, 2012; Hu An et al., 2017).

In order to create a new alfalfa cultivar at the Institute of Forage Crops, Pleven started breeding program, which base of the choice of mother plants in

(Kertikova, 2012),
(Kertikova, 2014).

condition of competition (dense sward). For this purpose, evaluation, selection and analysis of alfalfa cultivars at the regrowth rate (Kertikova, 2012), dry matter yield and persistence (Kertikova, 2014) were performed.

This article presents data on crude protein content in dry matter of alfalfa cultivars and its relationship to dry matter yield.

MATERIAL AND METHODS

During the period 2006-2012 nine alfalfa cultivars, registered in the Official Cultivar List (Bulgaria) were studied. The experimental material includes: Bulgarian alfalfa cultivars Pleven 6, Obnova 10, Dara, Dama, Prista 2, Prista 3, Prista 4, Mnogolistna 1 and French cv. Europe (European standard and the most propagated foreign cultivar in country).

The field trial was a randomized complete block design in four replication with 5 m² plot size, row to row space was 12,5 cm, and 3 g were seeded per m². Sowing was done by hand at depth of 2 cm. Lucerne is grown under non-irrigating conditions, and harvesting for forage is carried out at early flowering stage. The number of cuts and dates of harvesting are presented in Table 1.

1. 2006-2010
Table 1. Number of cuts and dates of harvesting during the period 2006-2010

/ Cut	2006	2007	2008	2009	2010
	04.07.	15.05.	08.05.	25.05.	18.05.
	08.08.	12.06.	09.06.	30.06.	14.07.
	19.10.	30.08.	09.07.	03.08.	19.07.
V	-	17.10.	11.08.	-	23.08.
V	-	-	23.10.	-	-

The cultivars included in the study are analyzed on a range of biological, economic, chemical characteristics of forage and persistence.

In the publication, the focus is on the crude protein content in the dry matter. Plant samples are unified in terms of morphology, which corresponds to the

60° . () Kjeldahl.
 2006-2010 . ()
 Excel 2003 (MS Office).

beginning of flowering and are dried at 60°C. Crude protein (CP) is determined by the Kjeldahl method. Data on crude protein content in dry matter for the period 2006-2010 (five consecutive years) of first cut, second cut including leaves and stems and average of all cuts by year and average over the period.

Statistical data processing was performed by means of Excel 2003 (MS Office).

RESULTS AND DISCUSSION

The first cut of alfalfa developed under the best supply of temperature and moisture and formed more than 60% of the annual yield. Therefore the data for protein content in the first cut is of special interest. The results are presented in Table 2. All cultivars recorded the highest values in the first year (average 21,03%) and this corresponds to the alfalfa biology. The data show cultivar variations, such as cv. Mnogolistna 1 and Obnova 10 being higher values 22,26% and 22,13%, respectively. The lowest is the CP of cv. Prista 2 – 19,79%, and the other cultivars occupy an intermediate position and are aligned by values.

60%
 21,03%
 22,26%
 22,13%
 2 – 19,79%
 2.
 (% . .)

Table 2. Crude protein content (% from dry matter) in forage mass (first cut) of alfalfa cultivars

/Cultivar	2006	2007	2008	2009	2010	/average
/Europe	20,61	17,74	17,08	16,04	19,22	18,13
6/Pleven 6	20,61	14,74	15,90	14,96	19,19	17,08
10/Obnova 10	22,13	15,45	16,77	14,31	18,88	17,50
/Dara	21,34	15,84	17,21	15,72	19,36	17,89
/Dama	20,28	15,80	17,08	14,43	19,12	17,34
2/Prista 2	19,79	15,49	16,33	14,52	19,37	17,10
3/Prista 3	20,80	16,59	16,39	16,24	20,11	18,02
4/Prista 4	21,45	15,53	15,75	15,62	19,21	17,51
1/Mnogolistna 1	22,26	18,12	17,61	17,35	19,56	18,98
/average	21,03	16,14	16,68	15,46	19,33	17,72

The results for the next three years of first cut in all cultivars show a lower crude protein content, such as

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- 14,31% 2009
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(% . .)

Mnogolistna 1 retaining a leader position, whereas for cv. Obnova 10 the lowest value was obtained in 2009 – 14,31%.

On average, for five years of first cut, the CP content is highest in the forage mass of Mnogolistna 1, Europe and Prista 3.

Data analysis for the second cut also shows (Table 3) that the cv. Mnogolistna 1 and Europe distinguished with highest CP content. It is noteworthy that in most cultivars the values in the second cut vary by year, whereas for the cv. Mnogolistna 1, the variation is weak or almost absent. In this respect, the variety differs significantly from the other cultivars included in the study.

Table 3. Crude protein content (% from dry matter) in forage mass (second cut) of alfalfa cultivars

/Cultivar	2006	2007	2008	2009	2010	/average
/Europe	21,42	16,26	19,30	22,76	22,73	20,49
6/Pleven 6	19,77	20,75	18,96	19,85	19,66	19,79
10/Obnova 10	18,96	20,48	19,71	20,93	20,94	20,20
/Dara	19,95	20,83	18,00	22,59	21,86	20,64
/Dama	20,07	19,90	17,50	22,16	18,13	19,55
2/Prista 2	19,08	15,20	19,90	20,01	24,04	19,64
3/Prista 3	19,09	17,19	19,27	21,51	20,99	19,61
4/Prista 4	19,29	18,19	19,13	22,82	20,75	20,03
1/Mnogolistna 1	20,98	22,21	20,07	22,52	22,94	21,74
/average	19,84	19,00	19,09	21,68	21,33	20,19

4.
(24,86%-32,41%),
(12,64%-16,62%).
20,86% 2 28,69%
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10 1
27,1% 27%.
6,
27,6%,

The results for the CP content in the leaves and stems on the second cut are presented in Table 4. The average values by year for the protein content of the leaves varies widely (24,86%-32,41%) than in the stems (12,64%-16,62%). The strongest differentiation between cultivars is reported in the second year. In the leaf fraction, the CP content varied from 20,86% for Prista 2 to 28,69% for Mnogolistna 1. The other cultivars in the study occupied an intermediate position, the closest to cv. Mnogolistna 1 being cultivars Pleven 6, Obnova 10 and Dara - 27,6%, 27,1% and 27%, respectively.

4.

(% . .)

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Table 4. Crude protein content (% from dry matter) in leaves and stems (second cut) of alfalfa cultivars

Cultivar	2006		2007		2008		2009		2010	
	leaves	stems	leaves	stems	leaves	stems	leaves	stems	leaves	stems
Europe	26,95	13,89	21,62	10,90	31,95	12,99	26,34	15,83	32,47	12,40
Europe 6	25,59	11,96	27,60	13,90	31,55	13,57	25,80	15,62	31,38	12,86
Pleven 6	23,67	12,25	27,10	13,87	31,72	12,29	25,35	17,89	31,95	13,64
Obnova 10	25,21	11,90	27,00	14,67	32,62	12,66	24,24	16,06	32,82	11,61
Dara	25,05	14,89	26,84	12,96	31,19	12,01	24,28	16,25	32,25	11,91
Dama	25,99	12,17	20,86	9,55	30,19	12,88	22,33	17,56	32,02	12,63
Prista 2	25,87	12,31	24,19	10,20	32,26	12,89	25,43	17,91	33,46	12,66
Prista 3	26,82	11,76	26,35	10,03	30,62	12,43	25,41	16,32	32,34	13,35
Prista 4	27,48	12,68	28,69	15,74	33,26	12,92	24,64	16,21	33,00	12,93
Mnogolistna 1	25,88	12,64	25,58	12,42	31,71	12,73	24,86	16,62	32,41	12,66
average										

15,74% (9,55% (2)
1).

14,89% (2006), 2007 (15,74%), 2008 (17,91%), 2009 (13,64%).

1

The stem fraction also observed significant differences between varieties, with CP ranging from 9,55% (Prista 2) to 15,74% (Mnogolistna 1).

It is found that during the years of study, different cultivars occupy the first position of protein content in the stems. For example: 2006 - Dama (14,89%), 2007 - Mnogolistna 1 (15,74%), 2008 - Pleven 6 (13,57%), 2009 - Prista 3 (17,91%) and 2010 - Obnova 10 (13,64%). Opposite in the leaves cv. Mnogolistna 1 has the highest CP content in four of the five years. The results obtained in a categorical manner show that the cv. Mnogolistna 1 has higher crude protein content in the dry matter. This is because the study has eliminated a major factor that influences - harvesting phase, respectively morphological stages. It is also established that the factor plant age does not indirectly affect the CP content in the leaf fraction. Variation of protein content in the stems of different cultivars may be

related to the sward thinning and the formation of thicker stems with more cell walls and less protein.

The average results of the cultivars by year and average of all cuts (Table 5) shows an alignment in the values for CP. An exception is the second year of alfalfa cultivation when the cultivars are formed three groups: in the first group is Mnogolistna 1 (22,13%), in the second group fall most cultivars with values from 19,3% to 20,6% and in the third group is cv. Europe (17,92%) and Prista 2 (18,31%).

(5) ,
1 (22,13%),
19,3% 20,6%
(17,92%) 2 (18,31%).

5.

(% . .)

Table 5. Crude protein content (% from dry matter) in forage mass of alfalfa cultivars, average from all cuts

/Cultivar	2006	2007	2008	2009	2010	/average
/Europe	22,79	17,92	21,34	19,82	20,50	20,47
6/Pleven 6	22,54	20,31	20,87	18,39	19,06	20,23
10/Obnova 10	23,74	20,20	21,60	18,78	19,41	20,74
/Dara	22,54	20,10	21,20	18,80	19,79	20,48
/Dama	23,16	20,60	20,94	18,89	18,87	20,49
2/Prista 2	22,00	18,31	21,18	18,25	20,21	19,99
3/Prista 3	22,99	19,36	21,13	19,17	19,68	20,46
4/Prista 4	23,41	19,46	20,80	19,66	19,67	20,60
1/Mnogolistna 1	23,50	22,13	22,38	20,89	20,65	21,91
/average	22,96	19,82	21,27	19,18	19,76	20,59

19
(21,91%),
(19,99%).
(2017),
(6) ,
(Tucak et al., 2008; Davodi et al., 2011; Marinova et al., 2014; Marinova, 2017),
- r -0,33.

Under the conditions of experience, an average of 19 cuts with the highest crude protein content is Mnogolistna 1 (21,91%), and the lowest cv. Prista 2 (19,99%). In a study of Marinova (2017) where cv. Prista 2 was used as a standard, also reported a similar value (19,36%) in the cultivar.

Data from the correlation analysis (Table 6) show that the dry matter yield is in negative correlation with the crude protein content. Similar results have been obtained from other authors (Tucak et al., 2008, Davodi et al., 2011, Marinova et al., 2014, Marinova, 2017), with prevailing conclusions that the relationship is weak - r to -0,33.

6.
() (DMY)
Table 6. Correlation coefficients between crude protein content () and dry matter yield (DMY)

Character	CP first cut	CP second cut	CP average 19 cuts	DMY first cut	DMY second cut	DMY average 19 cuts
CP first cut	-					
CP second cut	0,843**	-				
CP average 19 cuts	0,834**	0,854*	-			
DMY first cut	- 0,155	- 0,262*	- 0,064	-		
DMY second cut	- 0,331*	- 0,328*	- 0,326*	0,796**	-	
DMY average 19 cuts	- 0,309*	- 0,298*	- 0,298*	0,886**	0,969**	-

** , * significant differences between the level of a factor at P 0.01 and P 0.05, respectively

(r=0,843)
(r=0,834).
al., 2006),
(Krachunov et al., 2006),
2014)
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6,
1
19
(21,91%),

There is, however, a strong positive relationship between the CP content of first and second cuts (r = 0,843) and an average of 19 cuts (r = 0,834). This is confirmed the conclusion by another our study (Krachunov et al., 2006), that the data on forage quality obtained for the first cut, irrespective of the study year, could be used for forage quality estimation when comparing alfalfa cultivars.

The results presented in this article as well as those in previous for dry matter yield (Kertikova, 2014) give us reason to claim, that the alfalfa cultivars Pleven 6, Dara and Prista 3 have a good balance between yield and crude protein content in the dry matter and respectively with a higher yield of protein per unit area. We deemed that in breeding programs with aim to create a new cultivar with a higher crude protein content, cv. Mnogolistna 1 can be used both as a starting material and as a standard.

CONCLUSIONS

Under the test conditions, an average of 19 cuts with the highest crude protein content is cv. Mnogolistna 1 (21,91%), and the lowest cv. Prista 2

(19,99%).
 -
 (24,86%-32,41%),
 (12,64%-16,62%).
 (, 1,
 6, 3 10)
 1,
 -
 6, 3

(19,99%). By year the protein content in the leaves varies widely (24,86%-32,41%) than in the stems (12,64%-16,62%). Over the years, different cultivars (Dama, Mnogolistna 1, Pleven 6, Prista 3 and Obnova 10) occupy the first position of protein content in the stems, whereas in the leaves cv. Mnogolistna 1 has the highest content.

The dry matter yield is in negative correlation with the crude protein content. The alfalfa cultivars Pleven 6, Dara and Prista 3 have a good balance between yield and crude protein content in the dry matter.

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Chemical composition of perennial forage crops depending on the system of cultivation and correlative relationships with root pests damage

Ivelina Nikolova*, Natalia Georgieva, Viliana Vasileva

Institute of Forage Crops, 89 "General Vladimir Vazov" Str., 5800 Pleven, Bulgaria

*E-mail: imnikolova@abv.bg

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SUMMARY

Otiorrhynchus ligustici L.
Sitona
2015-2017.
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To determine the effect of the system of cultivation of perennial legume crops on their chemical composition and correlative relationships with damage from root pests of the *Sitona* genus and *Otiorrhynchus ligustici* L., a field trial was performed in the Institute of Forage Crops, Pleven, Bulgaria in the period 2015-2017. The study was done on the sainfoin, birdsfoot trefoil and alfalfa stands (grown alone) and in mixtures with cocksfoot sowed under cover of spring forage pea. It was found that the growing of forage crops in mixtures with cocksfoot has a positive effect on the chemical composition of aboveground and root biomass, increasing to varying degrees the crude protein, calcium and phosphorus content. Correlative relationships between the chemical composition of the aboveground and root

Otiorrhynchus ligustici L.

Sitona

- biomass with the main indicators
- characterizing the damage caused by weevils of *Sitona* genus and
- *Otiorrhynchus ligustici* L. were identified.

Key words: sainfoin, birdsfoot trefoil, alfalfa, system of cultivation, chemical composition

INTRODUCTION

Alfalfa as forages plays an important role in the diets of ruminants by providing protein, energy, minerals and vitamins. It produces more protein per hectare than any other crop for livestock and is an important source of vitamin A (Sottie, 2014). The nitrogen content of plants varies among varieties, and with cultivation and harvest management practices. The crowns of alfalfa are the main areas of nitrogen deposition in the plant and the nitrogen in the roots contribute significantly to plant regrowth after harvest.

Legumes, including alfalfa, grown with grasses offer several advantages over grasses grown alone. Baylor (1974) noted that including legumes usually results in increased yield, higher quality, and improved seasonal distribution of forage.

Legume-grass mixtures have reduced weed encroachment and erosion and have led to greater stand longevity than legume or grass monocultures (Droslom and Smith, 1976).

Popp et al. (2000) indicated that alfalfa can be used to maximize beef production on pastures as a monoculture or as a dominant species in a forage mixture.

The applying of polyculture farming models is also an important cultural practice on pest control. These models

(Risch, 2005; Moonen and Bàrberi, 2008).

(Nikolova et al., 2016; Nikolova et al., 2017).

(Newman et al., 2009).

(Sanderson, 2010; Woodward et al., 2013),

(Tooker and Frank, 2012).

- are based on the principle of reducing the density of the pests by increasing the species diversity (Risch, 2005; Moonen and Bàrberi, 2008).

- The reduced number of harmful insect species is associated with a reduction in their damage, which correlates with higher feed quality and increases its nutritional value (Nikolova et al., 2016; Nikolova et al., 2017).

- Feed quality is related to the content of nutrients, energy, proteins, digestibility, fiber, minerals, vitamins, and sometimes animal products (Newman et al., 2009). One of the factors influencing the quality of the feed is the system of cultivation of forage crops (Sanderson, 2010; Woodward et al., 2013) and the presence of insect pest damage (Tooker and Frank, 2012).

- The purpose of the study is to compare the chemical composition between perennial legume crops grown alone and in mixtures with cocksfoot as well as to determine correlative relationships with damage from root pests.

MATERIAL AND METHODS

The trial was performed in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria. Sainfoin, birdsfoot trefoil and alfalfa were sown alone (100%) and in mixtures with cocksfoot (50:50%) (Table 1). A randomized method is applied with a plot size of 10 m² (7.15 x 1.40 m) and a 4-fold repeat of the variants. Perpendicular to the main crops and mixtures in the first year (2014) spring forage pea was sown as a cover crop with 75% of sowing rate.

- In the stage of flowering-beginning of the pod formation of legume crops soil monoliths (20 x 200 x 40 cm) from the second cut were taken for a whole-plant

2015-2017
(100%)
(50:50%)
1).
(7.15 1.40 m) 4-
10 m²
75%
-
20 200 40 cm

() - , , : - / , (cm), (cm). 2016 2017 , 15 100 60 . (AOAC, 2001) () Kjeldahl (= N x 6.25), (), - (Sande, 1979). Anova , Tukey 5% (0,05). Statgraphics Plus (1995) Windows Ver. 2.1.

(with root and aboveground mass) harvesting of the plants. After washing with water the plants were carefully separated to preserve their integrity and nodule biomass.

The next indicators were recorded: nodulation (number of nodules per root), number of healthy and damaged nodules on root per plant, length of spiral furrows (cm), plant height (cm).

In order to determine the chemical composition of the aboveground and root mass in 2016 and 2017, the plant samples taken are fixed for 15 minutes at 100°C and dried to a constant weight in a 60°C in thermostat. The chemical composition is determined by standard methods of the Weende system (AOAC, 2001) and includes crude protein (CP) by Kjeldahl method (crude protein is calculated on the formulae CP = total N x 6.25), crude fibers (CF), phosphorus – colorimetrically by hydroquinone, calcium – complexometrically (Sande, 1989).

The mathematical processing of the data was done using an Anova for a one factor case, the mean being compared by a Tukey test in 5% provenance (P 0.05). A multi-factor regression analysis of Statgraphics Plus (1995) for Windows Ver. 2.1. was also used.

1.

Table 1. Variants of the experiment

Variant	Ratio, %	Sowing rate, kg/da
(<i>Onobrychis Adans.</i>) () Sainfoin (<i>Onobrychis Adans.</i>) (local population)	100%	12.0
(<i>Lotus corniculatus</i> L.) Birdsfoot trefoil (<i>Lotus corniculatus</i> L.) cv. Leo	100%	1.5
(<i>Medicago sativa</i> L.) 6 Alfalfa (<i>Medicago sativa</i> L.) cv. Pleven 6	100%	2.5
+ /Sainfoin + cocksfoot	50:50%	6.0 + 1.750
+ /Birdsfoot trefoil + cocksfoot	50:50%	0.750 + 1.750
+ /Alfalfa + cocksfoot	50:50%	1.750 + 1.750

RESULTS AND DISCUSSION

The results of the chemical analysis of the root mass of the perennial legume forage crops (sainfoin, birdsfoot trefoil and alfalfa), grown alone and in a mixtures

with cocksfoot showed that the dry matter content of the crops from the stands studied during the years and average for the period did not differ statistically and varies within narrow limits (92.3-93.9%) (Table 2). The crude protein (CP) ratio is different, with a maximum value for the alone grown legume crops being found in alfalfa at 102.8 g kg⁻¹ on average for 2016-2017 with significant differences to the other two cultures. The CP content in birdsfoot trefoil and sainfoin is by 9.0 and 19.3% lower.

2.

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Table 2. Chemical composition of root mass of forage legumes in pure and mixed stands with cocksfoot

/ I	Year	E / S	E+E S+C	/ BT	+ BT+C	/ A	+ A+C	/ C (+) / (S+C)	/ C (+) / (BT+C)	/ C (+) / (A+C)	/ C	LSD _{0.05%}
DM	2016	92.3 a	91.3 a	91.6 a	92.3 a	91.4 a	92.3 a	92.3 a	91.9 a	91.1 a	92.8 a	1.874
	2017	94.1 a	93.8 a	92.9 a	92.6 a	94.4 a	94.7 a	95.5 a	94.5 a	95.0 a	94.1 a	4.992
		93.2 a	92.6 a	92.3 a	92.5 a	92.9 a	93.5 a	93.9 a	93.2 a	93.0 a	93.5 a	2.757
CP	2016	86.4 e	98.0 f	109.1 i	104.0 g	107.3 h	113.9 j	47.7 b	52.0 c	77.0 d	37.8 a	1.194
	2017	79.6 de	80.8 e	78.1 d	95.3 f	98.3 g	99.9 g	43.3 b	44.0 b	62.9 c	36.0 a	1.897
		83.0 e	89.4 f	93.6 g	99.6 h	102.8 i	106.9 j	45.5 b	48.0 c	70.0 d	36.9 a	1.373
CV	2016	332.0 i	361.4 j	286.3 g	297.7 h	240.3 e	269.8 f	203.8 d	156.7 b	187.4 c	147.2 a	8.495
	2017	235.3 d	333.5 g	255.2 e	307.1 f	262.6 e	241.6 d	128.0 a	158.1 c	125.9 a	141.8 b	8.662
		283.6 f	347.4 h	270.7 e	302.4 g	251.4 d	255.7 d	165.9 c	157.4 b	156.7 b	144.5 a	5.573
	2016	1.131 f	1.247 g	1.031 e	1.268 h	0.776 a	0.942 d	0.919 c	0.913 c	0.908 c	0.804 b	0.017
	2017	1.351 de	1.486 f	1.252 c	1.320 d	1.252 c	1.388 e	1.018 b	1.205 c	1.586 g	0.778 a	0.047
		1.392 g	1.468 i	1.136 e	1.176 h	1.279 c	1.480 f	0.826 b	0.958 d	1.106 g	0.791 a	0.022
	2016	0.218 ab	0.251abc	0.234abc	0.324bcd	0.175 a	0.320bcd	0.276abc	0.382 d	0.328 cd	0.312bcd	0.102
	2017	0.149 a	0.175 a	0.107 a	0.188 a	0.206 a	0.221 a	0.147 a	0.152 a	0.180 a	0.275 a	0.201
		0.169 ab	0.204 ab	0.163 a	0.204 ab	0.192ab	0.224 ab	0.258 ab	0.205ab	0.236 ab	0.294 b	0.119

Legend: I-Indicator; DM-Dry Matter, %; CP- Crude protein, %; CF- Crude fiber, g kg⁻¹; Ca-calcium, g kg⁻¹; P- Phosphorus, g kg⁻¹; -average; S-sainfoin; S+C- sainfoin by the mixture of the sainfoin + cocksfoot; BT- birdsfoot trefoil; BT+C- birdsfoot trefoil by the mixture of the birdsfoot trefoil+ cocksfoot; A-alfalfa; A+C-alfalfa by the mixture of the alfalfa+cocksfoot; C (S+C)-cocksfoot by the mixture of the sainfoin+cocksfoot; C (BT+C)-cocksfoot by the mixture of the birdsfoot trefoil +cocksfoot; A (A+C)-alfalfa by the mixture of the alfalfa+cocksfoot

(Avicé et al., 1997)

Crude protein in the roots plays a major role in the formation of the regrowths (Avicé et al., 1997) and is an

4.0% (+)
7.7% (+)
89.6%
(30.1%)
(23.3%)
()
(283.6 g kg⁻¹) - 4.5
11.4%
(22.5%),
(11.7%)
(1.7%)
(Pavlov,
1996),
(r = -0.531).
8.4 ()
14.8% ()
).

indicator of the potential protein nutrition of the forages. Its content varies depending on the model of the cultivation of forage crops and its inclusion in two components mixtures with cocksfoot results in a significant increase in protein values of 4.0% (alfalfa + cocksfoot) to 7.7% (sainfoin + cocksfoot). Significantly more pronounced is the increase of the CP in the grass component in mixtures compared to its alone growing. With the greatest increase in the value of 89.6% the mixture between alfalfa and cocksfoot was distinguished, followed by birdsfoot trefoil and cocksfoot mixture (with a 30.1% increase) and sainfoin and cocksfoot mixture (23.3%) compared to the grass stand.

The crude fiber content of the legume crops was found to be significant the highest in the sainfoin stands (283.6 g kg⁻¹ on average) and was by 4.5 and 11.4%, respectively lower in birdsfoot trefoil and alfalfa. The increase in the values of the indicator in the mixtures follows a similar tendency, with the strongest increase in CF content being found in mixtures of sainfoin and cocksfoot (by 22.5%), followed by birdsfoot trefoil and cocksfoot mixtures (by 11.7%) and alfalfa and cocksfoot mixtures (by 1.7%) versus alone grown legume stands. It is known that there is a negative correlative relationship between the crude protein and crude fiber content (Pavlov, 1996), which is confirmed by the our study (r = -0.531). In the grass component grown in a mixture as compared to alone grown grass the CF content grew to a lesser extent from 8.4 (alfalfa and cocksfoot) to 14.8% (sainfoin and cocksfoot).

Calcium plays an important role in the growth of the root system in the plant organism, therefore the need for it is manifested by the germination of seeds. In addition, the element facilitates the absorption of nitrogen and is critical to the photosynthesis process, as well as takes a part in other physiological processes

(Acharya et al., 2012)

(Russell, 2002).

3.5, 5.4 15.7%

21.1 25.5%

16.4,

4.5-39.8%,
12.4 30.3%

Otiorrhynchus ligustici L.,

Sitona

(Nikolova et al., 2018).

Zhekova, 2018).

(Petkova et al., 2005;

114.6 g kg⁻¹,

104.1

(3).

important to the plant. Phosphorus is one of the main elements that are inaccessible to crop plants (Acharya et al., 2012) and often restrict the growth and development of plants (Russell, 2002). In the present study, the content of calcium and phosphorus macro elements in the legume component also increases in mixtures relative to pure crops, but to varying degrees. Calcium content was found to increase significant on average by 3.5, 5.4 and 15.7%, respectively in mixtures of birdsfoot trefoil, sainfoin and alfalfa. Contrary to the phosphorus content, this increases with 16.4, 21.1 and 25.5%, respectively in mixtures of alfalfa, sainfoin and birdsfoot trefoil.

With respect to the grass component, the calcium content increases in the range of 4.5-39.8% and the phosphorus decreases from 12.4 to 30.3% in the mixtures compared to the pure grass stand.

In a previous study it was found that the system of cultivation of legume crops influenced the preferences of the soil pests - the species of *Sitona* genus and *Otiorrhynchus ligustici* L. and in the mixtures the damage was reduced as compared to pure ones (Nikolova et al., 2018). On the other hand, some authors report that in the case of minimal or lack of damage on the roots of alfalfa from soil insects (lucerne longicorn), the content of crude protein, crude fiber, calcium and common phenols increases and the content of phosphorus, magnesium and sugars slightly decreases or does not respond (Petkova et al., 2005; Zhekova, 2018). Probably one of the reasons for the improved chemical composition of plant roots in mixtures is the lesser degree of damage from soil root pests.

The aboveground mass of forage legumes has high crude protein content ranging from 104.1 to 114.6 g g kg⁻¹ and the alfalfa was distinguished with significant the highest value (Table 3). Sainfoin and birdsfoot trefoil stands

7.6 9.1% -
 2.7, 7.6 9.2%
 6.3 25.4%
 (6.1%).
 3.

showed lower crude protein content by 7.6 and 9.1%, respectively. The applying of the two component model of cultivation has a significant positive effect on the chemical composition of the individual components, increasing the CP content by 2.7, 7.6 and 9.2%, respectively, in the case of birdsfoot trefoil, sainfoin and alfalfa in mixtures. Regarding the cocksfoot the values of this indicator increased by 6.3 and 25.4% in mixtures with birdsfoot trefoil and alfalfa but decreased in the case of the sainfoin and cocksfoot mixtures (by 6.1%).

Table 3. Chemical composition of aboveground mass of forage legumes in pure and mixed stands with cocksfoot

I	Year	E/S	E (E+E) S (S+C)	/BT	(+) BT (BT+C)	/A	(+) A(A+C)	/C (+) /(S+C)	/C (+) /(BT+C)	/C (+) /(A+C)	/C	LSD _{0.05%}
DM	2016	93.7 a	94.1 a	94.0 a	93.9 a	94.1 a	94.0 a	94.4 a	94.2 a	93.3 a	94.5 a	3.128
	2017	93.5 a	95.4 a	94.5 a	93.3 a	94.9 a	93.2 a	94.5 a	94.9 a	94.0 a	93.9 a	2.824
		93.6 a	94.7 a	94.2 a	93.6 a	94.5 a	93.6 a	94.4 a	94.5 a	93.6 a	94.2 a	2.154
CP	2016	110.3 d	120.2 g	115.4 e	117.5 f	118.0 f	134.0 h	80.4 a	90.3 b	107.4 c	81.2 a	1.453
	2017	101.5 d	107.7 e	92.9 c	96.3 c	111.1 e	116.2 f	70.0 a	80.0 b	93.6 c	79.0 b	4.877
		105.9 f	114.0 g	104.1 e	106.9 f	114.6 h	125.1 i	75.2 a	85.2 c	100.5 d	80.1 b	2.648
CV	2016	291.2 b	301.5 c	355.0 d	370.8 e	349.3 d	372.3 e	259.7 a	258.9 a	285.9 b	274.8	5.889
	2017	261.9 b	278.3 c	333.6 d	356.3 e	333.8 d	340.4 d	229.1 a	235.8 a	253.0 b	264.6	9.090
		276.5 c	289.9 d	344.3 e	363.6 h	341.5 e	356.3 f	244.4 a	247.3 a	269.4 b	269.7 b	6.163
	2016	1.449 e	1.439 e	1.020 c	1.026 c	1.307 d	1.571 f	0.640 a	0.710 b	0.629 a	0.628 a	0.026
	2017	1.311 d	1.351 e	1.025 c	1.039 c	1.418 f	1.444 f	0.613 a	0.683 b	0.695 b	0.600 a	0.035
		1.380 ef	1.395 f	1.022 d	1.033 d	1.362 e	1.508 g	0.626 a	0.696 c	0.662 b	0.614 a	0.022
	2016	0.188 a	0.233 d	0.218 b	0.220 bc	0.178 a	0.226 cd	0.368 h	0.258 e	0.291 f	0.306 g	0.009
	2017	0.170 a	0.219 cd	0.210 c	0.227 d	0.191 b	0.210 c	0.337 h	0.240 e	0.255 f	0.286 g	0.012
		0.179 a	0.226 c	0.214 b	0.223 bc	0.184 a	0.218 bc	0.353 g	0.249 d	0.273 e	0.296 f	0.010

Legend: I-Indicator; DM-Dry Matter, %; CP- Crude protein.; CF- Crude fiber, g kg⁻¹;Ca-calcium, g kg⁻¹; P- Phosphorus, g kg⁻¹; -average; S-sainfoin; S+C- sainfoin by the mixture of the sainfoin + cocksfoot; BT- birdsfoot trefoil; BT+C- birdsfoot trefoil by the mixture of the birdsfoot trefoil+ cocksfoot; A-alfalfa; A+C-alfalfa by the mixture of the alfalfa+cocksfoot; C (S+C)-cocksfoot by the mixture of the sainfoin+cocksfoot; C (BT+C)- cocksfoot by the mixture of the birdsfoot trefoil +cocksfoot; A (A+C)-alfalfa by the mixture of the alfalfa+cocksfoot

Similar results for improved forage quality in grass-legume mixtures vs. pure crops reported by Sleugh et al. (2000).

Sleugh et al. (2000).	According to the authors, the concentration of crude protein in smooth brome grass in mixtures with birdsfoot trefoil, alfalfa and <i>Trifolium ambiguum</i> is found higher by 31, 46 and 46%, respectively than that of the grass component which is an indicator of improved quality composition.
<i>Trifolium ambiguum</i> -	When comparing the average values of crude fiber content we found that sainfoin stands are distinguished with significant lower content (105.9 g kg ⁻¹), whereas the values for the other two crops were similar.
31, 46 46%	
(105.9 g kg ⁻¹),	
4.3, 4.8 5.6%	There is an increase of 4.3, 4.8 and 5.6% in the CF content in alfalfa, sainfoin and birdsfoot trefoil in mixtures with grass component. It should be noted that the CF content of cocksfoot in mixtures decreases from 0.1 (alfalfa + cocksfoot) to 9.4% (sainfoin + cocksfoot), a tendency not found at the root mass.
(+) 9.4%	
(+),	
(1.380 1.362 g kg ⁻¹),	The mineral composition of the crops studied is different with significant higher calcium content distinguishing sainfoin and birdsfoot trefoil (1.380 and 1.362 g kg ⁻¹ , respectively) and with significant higher concentration of phosphorus (0.214 g kg ⁻¹). In mixtures an improvement of the mineral composition of legume plants was observed and calcium content increased significant in alfalfa + cocksfoot mixtures by 10.7%, and the increase in sainfoin and birdsfoot trefoil mixtures was insignificantly (by 1.1 and 1.0%, respectively). With regard to the grass component a significant increase is only found in the birdsfoot trefoil mixtures (13.4%).
- (0.214 g kg ⁻¹).	
10.7%,	
(1.1 1.0%).	
(13.4%).	
(26.1%),	With a higher concentration of phosphorus in the legume component compared to the monoculture was the mixture of sainfoin (by 26.1%) followed by that of alfalfa (by 18.3%). The increase in the birdsfoot trefoil + cocksfoot variant was insignificantly (by 4.3%).
(18.3%).	
(4.3%).	

	-	Increased phosphorus content of the grass component in mixtures with significant difference compared to the pure grass stand is found only in the sainfoin + cocksfoot (by 19.1%).
(19.1%).	+	
<i>Sitona</i>	-	The weevils of the <i>Sitona</i> genus and alfalfa snout beetle damage the roots of forage legumes breaking the integrity of the root system by gnawing spiral furrows and holes. In addition, the larvae of <i>Sitona</i> weevils destroy the root nodules. It is this damage that affects the biochemical composition of plants by reducing the content of crude protein (Nikolova et al., 2015).
(Nikolova et al., 2015).	-	In the present study, the dependence between the indicators of damage from the root pests and the biochemical composition is expressed by correlation analysis (Table 4). The results show that the gnawing of the root nodules has an adverse effect on the biochemical composition with a moderate and strong negative correlation with the CP, calcium and phosphorus content in the root mass ($r = -0.638$, $r = -0.542$ and $r = -0.988$). Analogous is the tendency in the aboveground mass of plants with a strong negative dependence on CP content ($r = -0.716$) and for other biochemical indicators the correlation is mean with a negative sign.
(4).	,	
($r = -0.638$, $r = -0.542$ $r = -0.988$).	-	
($r = -0.716$).	-	
	-	Length of the gnawed furrows is negatively moderate or strongly dependent on the content of the presented indicators, with a stronger dependence being found in the biochemical composition of the root mass. The direct damage from the alfalfa snout beetle expressed as the length of the furrows is in the mean negative correlation with the content of CP, CF and phosphorus. This insignificantly effect on the calcium content both, in the roots and in the aboveground mass of plants.
	-	Length of the gnawed furrows

(r = - 0.440 r = - 0.896).

affects the level of CF and phosphorus in the aboveground mass by expressing an average and strong negative correlative relationship (r = - 0.440 and r = - 0.896, respectively).

The height as a morphological indicator is related to the biochemical composition of the plants with a positive correlation with the CP and calcium content and negative with the CF content at the root and aboveground mass. For the phosphorus element, the tendency is not clear.

4. Table 4. Correlative relationships (r)

/Indicators		/Root mass			
		/CP	/CF	Ca	
%	/% damaged nodules	-0.638	-0.062	-0.542	-0.988
	/Length of furrows	-0.341	-0.454	-0.017	-0.661
	/Height	0.508	-0.404	0.729	0.614
		/ Aboveground mass			
%	/% damaged nodules	-0.716	-0.345	-0.498	-0.413
	/Length of furrows	-0.020	-0.440	0.240	-0.896
	/Height	0.956	-0.047	0.890	-0.207

kg⁻¹ : - , g kg⁻¹; - , g kg⁻¹; - , g kg⁻¹; - , g

Legend: CP- Crude protein, g kg⁻¹; CF- Crude fiber, g kg⁻¹;Ca-calcium, g kg⁻¹; P- Phosphorus, g kg⁻¹

CONCLUSIONS

— Growing of sainfoin, birdsfoot trefoil and alfalfa in mixtures with cocksfoot has a positive effect on the chemical composition of the aboveground and root biomass, increasing to varying degrees the content of crude protein, crude fiber, calcium and phosphorus. The content of crude protein in root mass of legume component in mixtures increases from 4.0% to 7.7%, the macro elements calcium and phosphorus – in the range 3.5-15.7% and 16.4-25.5% respectively, and in the grass component the crude protein increases from 23.3 to 89.6%.

— The applying of the two component model of cultivation has a positive effect on the chemical composition of the aboveground mass,

4.0% 7.7%,
3.5-15.7% 16.4-25.5%
23.3 89.6%.

		2.7	9.2%,
-	6.3	25.4%.	
-			
			<i>Sitona</i>
			<i>Otiorrhynchus ligustici</i> L.

increasing the CP content in legume component from 2.7 to 9.2% and in the grass component from 6.3 to 25.4%. An improved mineral composition of the legumes was found in the mixtures.

Negative correlative relationships between the chemical composition of the aboveground and root biomass with the main indicators characterizing the damage caused by weevils of *Sitona* genus and *Otiorrhynchus ligustici* L. were identified.

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Comparative characteristics of the composition and enzyme digestibility of temporary and permanent pasture

Viliana Vasileva, Ina Stoycheva*, Yordanka Naydenova

Institute of Forage Crops, 89 Gen. Vladimir Vazov Str., 5800 Pleven, Bulgaria

*E-mail: ina7777@abv.bg

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SUMMARY

During the pasture season, the permanent pastures are mainly used, but their yield and nutritional composition in most cases do not cover the needs of the higher dairy animals. A good alternative is temporary pastures with a controlled grass and legume composition and a high nutritional value, to which there is a high interest from farmers in the recent years. The purpose of study is an establishment and comparison of the changes in chemical composition, plant cell walls fiber components and enzyme digestibility of the forage from first growth of temporary and permanent pastures. In 2017, in April, in the vegetation of first pasture growth during four 7 day week intervals, the changes in chemical composition and *in vitro* enzyme digestibility of pasture swards are studied. The average crude protein content is approximately the same in both pastures (12,39% and 11,62%) and shows a tendency to decrease from the beginning to the end of the period. The rate of change in CF content is dynamic in both

28,0% ()
32,6%
46,67 58,16%, ()
3,57 %-
47,07% 57,78%,
3,86 %-
3,30 %- ()
3,63 %-
0,974 %-
0,926%-
21,60% 26,70% 4
in vitro

grasses which increase by 28,0% and 32,6% in temporary and permanent pasture, respectively. NDF content grew from 46,67 to 58,16%, or an average of 3,57% units per week in natural pasture, and from 47,07% to 57,78%, or by 3,86% units per week in temporary pasture. The ADF content of sown pasture increases by 3,30% per week, while in natural pasture the growth is 3,63% per week.

Lignin content in permanent pasture increases by 0,974% units per week, while growth of temporary pasture is more higher by 17,5% and increased by 1,18%, respectively, over a period of 4 weeks. The reduction in digestibility of dry matter in temporary pasture is lower than in the permanent pasture, with the results obtained are – 21,60% and 26,70% respectively, over a period of 4 weeks.

Key words: temporary pasture, permanent pasture, chemical composition, plant cell walls fiber, *in vitro* digestibility

INTRODUCTION

The specific structure of the digestive system in ruminants allows efficient use of the green and roughage, and good absorption of nutrients in them. The main and indispensable forage for these animals during the spring-summer period are the permanent and/or temporary pastures, and during the winter period they mainly rely on the grass of natural and sown meadows (Kirilov et al., 2007). Green forage and hay vary considerably in terms of quality depending on the grass species and the plant development phase as well as the share of legume crops in.

Grazing is mainly done on natural pastures that occupy 30% of the agricultural area in the country (Kirilov and Mihovski, 2014). During the pasture season, it is mainly relied on natural pastures, but their yield and nutritional composition in most cases do not cover

(Kirilov et al., 2007).
30% (Kirilov and Mihovski, 2014).

(Vasilev et al., 2005; Vasilev, 2008; Kirilov and Vasilev, 2007).

(Graves et al., 2012; Stoycheva, 2015).

(Kirilov, 2010; Vasileva and Ilieva, 2016), (Radovic et al., 2003; Chourkova, 2010).

(Vu kovi , 2004),

(Ilieva and Kyuchukova, 2009; Chourkova, 2012).

(Naydenova and Pavlov, 2001, 2005ab; odorova and Kirilov, 2004; Bovolenta t al., 2008).

the needs of the more high productive animals. A good alternative is grassland with a controlled crop of grass and legume components and a high nutritional value, to which there is a growing interest from farmers in recent years. The most common are the annual grasses, legumes and perennial grass-legumes (Vasilev et al., 2005; Vasilev, 2008; Kirilov and Vasilev, 2007). Suitable for lactating sheep are pastures with a higher share of legume crops. Benefits based on such grass mixtures ensure high milk productivity (Graves et al., 2012; Stoycheva, 2015).). In our conditions, the grass mixtures with participation of Cocksfoot and Sainfoin are suitable (Kirilov, 2010; Vasileva and Ilieva, 2016) and the Birdsfoot Trefoil (Radovic et al., 2003; Chourkova, 2010). The Birdsfoot Trefoil survives trampling and is well grown after cutting (Vu kovi , 2004), and its feed biomass is high in crude protein and good digestibility of dry matter (Ilieva and Kyuchukova, 2009; Chourkova, 2012).

Feed quality correlates positively with its dynamically variable factors during vegetation: chemical composition, structural fiber content and digestibility, which determine the nutritional value of pastures (temporary or natural). The resulting animal production (milk, meat, wool) in turn depends on the quality of the green or canned forage. The need of such studies is also reinforced by the fact that during the pasture period the sheep have high productivity and high demands on the energy and protein to cover their needs. As the vegetation progresses, the chemical composition of the grasses changes and the nutritional value of the grasses decreases (Naydenova and Pavlov, 2001, 2005ab, Todorova and Kirilov, 2004, Bovolenta et al., 2008). For this period there are not enough comparative studies of the composition and digestibility of permanent and temporary pastures. They would give more clarity to optimize nutrition in order

in vitro

(),
(.)
(43° 23'N, 24° 34'E, 230 m
).
2014 .
(*Dactylis
glomerata* L.)
(*Lotus corniculatus*
L.), 50%
2017 .

in vitro

Retsch SM 100
1 mm.

Weende

(, 2000):
(/CP) *Kjeldahl* (-ISO
5983) (/CF) (
, 2007);
: /Neutral-
detergent fiber (/NDF)/,
(/ADF)/ /Acid-detergent fiber
/Acid-detergent lignin (/ADL)/

*Goering
and Van Soest* (1970) (EN ISO13906, 2008).
in vitro
(/IVDMD) (/IVOMD)
-
-
Aufrere (Todorov et
al., 2010).

to fully satisfying the energy and nutritional needs of the animals.

The purpose of this study was to compare the changes in the chemical composition and the in vitro enzyme digestibility in the process of vegetation of the first growth of the permanent and temporary pasture.

MATERIAL AND METHODS

The object of this study were natural and temporary pastures owned by the Institute of Forage Crops - Pleven, Bulgaria. The temporary pasture was sown in 2014 of Cocksfoot (*Dactylis glomerata* L.) and Birdsfoot Trefoil (*Lotus corniculatus* L.), 50% of the sowing rate for each species.

In 2017, samples of both pastures were given from Mid-April in 7 days to determine changes in composition and *in vitro* digestibility. The dried samples were milled by *Retsch SM 100* mill through a 1 mm mesh size.

For each sample, the chemical composition according to the commonly agreed *Weende* Method (AOAC, 2007) was determined: Crude Protein (CP) by *Kjeldahl* (BDS-ISO 5983) and Crude Fibers (CF) (AOAS, 2007); Structural carbohydrates or cell wall components: Neutral-detergent fiber (NDF), Acid-detergent fiber (ADF), Acid-detergent lignin (ADL) as a percentage of the dry matter of the feed were determined by the method of *Goering and Van Soest* (1970) (EN ISO13906 2008).

The *in vitro* enzyme digestibility of the dry (IVDMD) and organic (IVOMD) matter is determined as a percentage by *Aufrere* two-step pepsin-cellulose enzyme method by *Aufrere* (Todorov et al., 2010).

The test data was statistically processed, taking into account the mean (x) and its

()
 MS Office 2007.
 t-test ()
 > 0,05.

error with the application of the statistical program MS Office 2007. The validity of the difference between the values was determined by applying t-test (by Student) and a confidence level P > 0,05.

RESULTS AND DISCUSSION

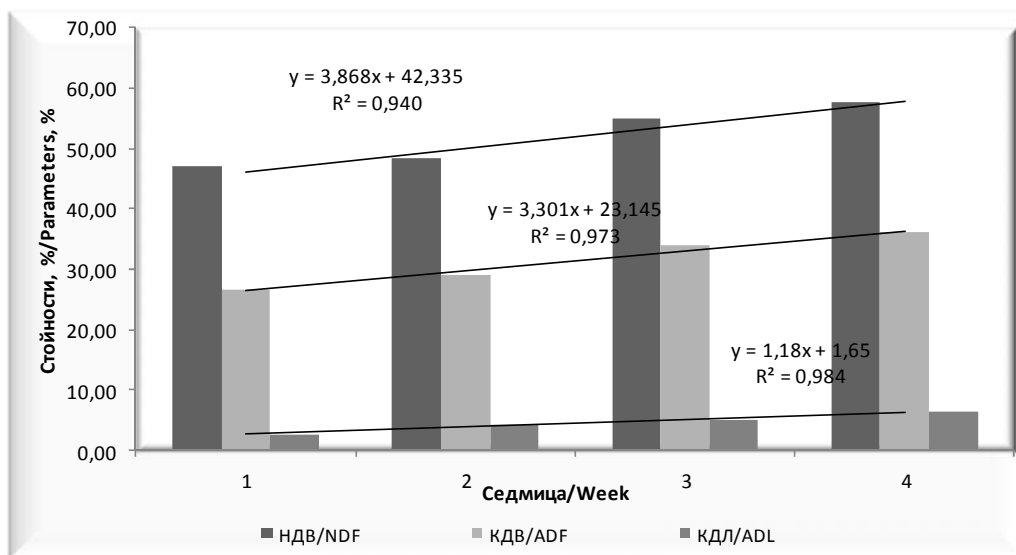
a 1
 11,62%)
 (12,39%)
 28,0%
 32,6%
 1.
 , %

Table 1 presents the data of changes in the chemical composition of biomass at first growth of permanent and temporary pastures. The average CP content is approximately the same for both pastures (12,39% and 11,62%) and shows a tendency to decrease from the beginning to the end of the period. The rate of change in CF content is dynamic in both pastures and increases by 28,0% in sown pasture and 32,6% in natural pasture, respectively.

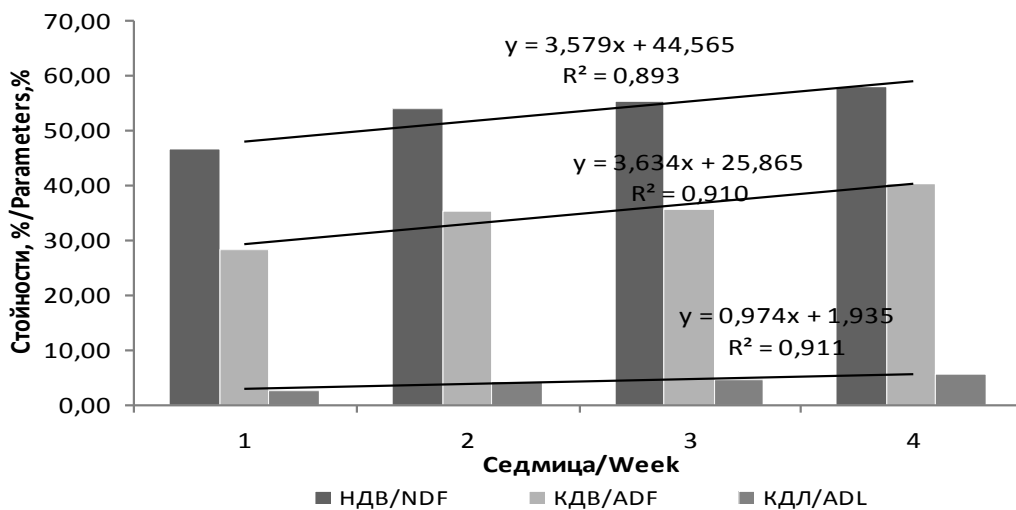
Table 1. Chemical composition of temporary and permanent pasture, % of Dry matter

/ Weeks		Development stage	CP	CF
2017				
/ Temporary pasture				
1 -15.04.		Without generative stems	13.06	17.74
2		Without generative stems	11.78	19.07
3		With generative stems	11.54	22.19
4		Beginning of flowering	10.11	24.66
/ Mean			11.62±1.04	20.92±2.70
/ Permanent pasture				
1 -15.04.		Without generative stems	15.13	16.96
2		Without generative stems	13.41	20.98
3		With generative stems	10.40	23.88
4		Grasses-earring	10.61	25.15
/ Mean			12.39±0.92	21.74±3.13

1 2. The changes observed in the content of structural carbohydrates are presented in Figure 1 and 2. It has been found that as the vegetation progresses and growing old of plants, the content of NDF, ADF and lignin increases, these trends being described by the corresponding linear equations.



1. 4
Fig. 1. Plant cell walls fiber components content in 4 weeks of temporary pasture



2. 4
Fig. 2. Plant cell walls fiber components content in 4 weeks of permanent pasture

()
 46,67 58,16%, 3,57
 %- (R² = 0,893)
 47,07%
 57,78%, 3,86 %-
 (R² = 0,940)
 ()
 26,64%
 36,00%, 3,30%-
 (R² = 0,973),
 28,47% 40,42%,
 3,63 %- (R² = 0,910)
 , 0,974 %-
 (R² = 0,911),
 17,5%
 1,18%-
 (R² = 0,984).
 4
 -
 (52,01%),
 (53,51%),
 31,40 34,95%.
 -
 (4,37%)
 (4,60%).
 -
 (odorova
 and Kirilov, 2002; Jochims et al., 2013).
 -
 (Naydenova and
 Pavlov, 2001; 2005b; odorova and

Over a period of four weeks, neutral detergent fiber (NDF) content increased from 46,67 to 58,16%, or 3,57% on average (R² = 0,893) weekly in natural pasture, and from 47,07% to 57,78% or 3,86 % units (R² = 0,940) per week at rest.

The content of acid-detergent fiber (ADF) from first to fourth weeks in sown pasture ranges from 26,64% to 36,00%, or on average increases by 3,30% units per week (R² = 0,973), and in natural pasture the values are from 28,47 % to 40,42%, or growth by 3,63% units (R² = 0,910) per week.

Lignin content grows similar to natural pasture by 0,974% units per week (R² = 0,911), while in sown pasture the growth is 17,5% higher and increases by 1,18% units (R² = 0,984).

The mean content of NDF in the 4-week period is slightly lower for temporary pasture (52,01%), compared to the permanent (53,51%) observed as a dependence and at the average for the period of ADF, from 31,40 to 34,95 %. Mean values of lignin were lower in natural pasture (4,37%) and higher in sown pasture (4,60%).

At the first growth of the natural and temporary pasture are observed the usual trends of changes in the chemical composition, related to the stage of grass growth (Todorova and Kirilov, 2002, Jochims et al., 2013).

The composition and nutritional value of grasses changes during the growth period. There is a constant tendency of decreasing the nutritional value with the progression of vegetation (Naydenova and Pavlov, 2001; 2005b, Todorova and Kirilov, 2002, Naydenova et al., 2003,

Kirilov, 2002; Naydenova et al., 2003; Bovolenta et al., 2008).

Bovolenta et al., 2008).

(3).
 1 2).
 57,22%, 72,93%
 21,6%,
 75,70% 54,74%,
 26,7% 4
 (26,4%),
 (21,8%).

As the vegetation progresses, the digestibility of both pastures decreases (Figure 3). The enzyme digestibility of dry and organic matter decreases in the direction from the first to the fourth week (Figures 1 and 2). IVDMD values decrease from 72,93% to 57,22%, or 21,6% on average, while in natural pasture, from 75,70% to 54,74%, or on average by 26,7% over a 4-week period.

A similar trend was observed in IVOMD, which was found to decrease at a faster rate in natural pasture (26,4%), compared with that of temporary (21,8%).

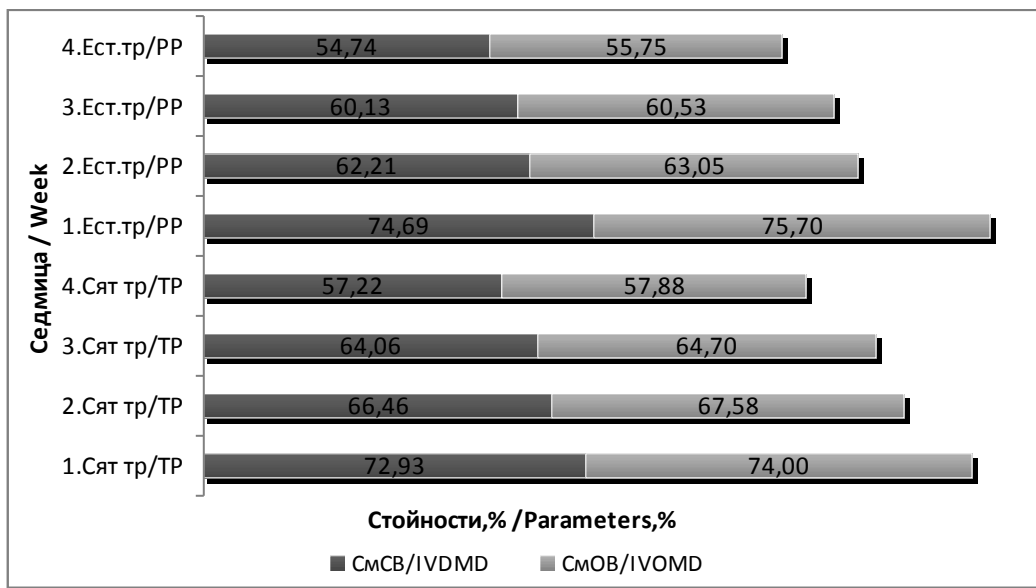


Fig. 3. IVDMD and IVOMD in 4 weeks of temporary (TP) and permanent pasture (PP)

Safari et al. (2011),
 68% 40%
 . Askar et al. (2014)

Our results correspond to the results of Safari et al. (2011), according to which the aging of plants the digestibility of the grass decreases from 68% to 40% at the end of the pasture period. Askar et al. (2014) found that the

73,4%

53,8%
26,7%.

Kirilov (2010),

Pavlov (1996)

(Vasilev, 2008; Stoycheva, 2015; Stoycheva et al., 2017).

2017 ()

in vitro

4

❖

(12,39% 11,62%).

❖

28,0%

32,6%

4

❖

digestibility of pastures decreased from 73,4% at the beginning of grazing to 53,8% at the end of the period or an average of 26,7%. The results obtained are not unexpected in view of the biological features of legumes and grasses crops. In legumes, there is a faster development after growing at the first growth compared to grasses.

In support of this finding are the results of Kirilov (2010), wh also observed the lower durability of legumes in mixtures with grasses. According to Pavlov (1996), the legumes shows a smaller competitive power, which explains the approximate digestibility of both types of pastures.

Decreasing the share of the legume component in grass mixtures with grasses has also been observed in studies by many authors (Vasilev, 2008, Stoycheva, 2015, Stoycheva et al., 2017). Participation of the legume component in both pastures (natural and sown) in 2017 is close. This made the nutritional value of the pastures approximately the same and the amount of crude protein assumed during the study period.

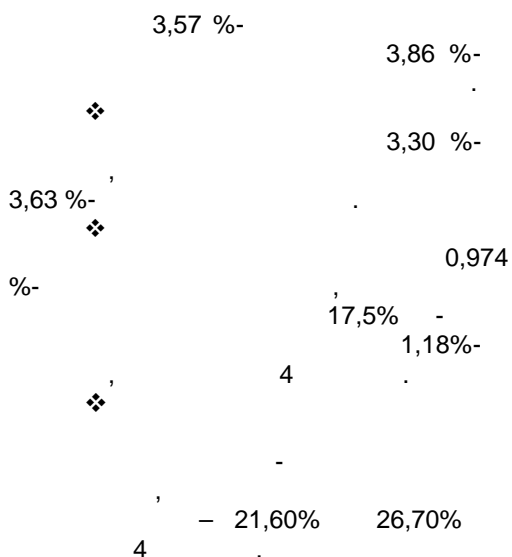
CONCLUSIONS

The results obtained in determining the changes in composition, plant cell walls fiber components and *in vitro* enzyme digestibility from first growth of natural and temporary pasture allow the following conclusions to be drawn:

❖ The mean CP content is approximately the same for both grasses (12,39% and 11,62%).

❖ The rate of change in CF content increased by 28,0% for temporary pasture and by 32,6% for natural pasture for a period of 4 weeks.

❖ The NDF content



increases by 3,57% units per week in natural grassland and by 3,86% units in temporary pasture per week.

❖ The ADF content in temporary pasture grows by 3,30% and in natural pasture by 3,63% per week.

❖ The lignin content increases by 0,974% in permanent pasture per week, while in the temporary pasture the growth is 17,5% higher and increases by 1,18% units for a period of 4 weeks.

❖ The reduction in the digestibility of dry matter in temporary pasture is lower than in the natural pasture, the results obtained being 21,60% and 26,70% respectively over a period of 4 weeks.

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Total Care

(*Medicago sativa* L.)

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Effect of foliar fertilization with Total Care on some morphological traits and forage productivity in Prista 5 alfalfa (*Medicago sativa* L.) variety

Diana Marinova*, Iliana Ivanova

Institute of Agriculture and Seed Science "Obraztsov chiflik" – Rousse, Bulgaria

*E-mail: diana27hm@abv.bg

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SUMMARY

- The aim of the present study was to
- determine the effect of Total Care organic mineral product application on the main morphological traits – natural plant height and grass stand density, expressed by stem number per m² and on the forage productivity in Prista 5 alfalfa variety.

The study was carried out from 2014 to 2017 at the experimental field of Institute of Agriculture and Seed Science "Obraztsov chiflik" - Rousse. The experiment included a control (untreated variant) and variant with Total Care product application. Alfalfa was grown without irrigation and green mass harvesting was carried out in early flowering stage.

The summarized results showed a different effect of Total Care, on the morphological traits studied and on the forage productivity, both in regrowths and

Total Care
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5.
2014-2017 .
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Total Care

Total Care

a

years. Total Care foliar fertilizer stimulated the development of significantly higher plants and had a slight positive impact on potential for stem formation. The foliar application of Total Care had a clearly expressed positive effect on dry matter yield. It was found that the stimulating effect of the product on the productive potential decreased in each subsequent growing season. The obtained results give a reason to consider that the phenotypic expression of plants height and stems number morphological traits and of the genetic ability for productivity is a result of the complex relationship genotype x foliar fertilization x environment conditions.

Key words: alfalfa, foliar fertilization, morphological traits, dry matter yield

INTRODUCTION

The alfalfa (*Medicago sativa* L.), is the most important forage legume widely grown throughout the world (Bouton, 2012). In Europe alfalfa is considered to be the species with the greatest contribution to sustainability of the agriculture for many reasons: nitrogen fixation, enrichment the soil fertility due to deep reaching root systems, quick decompose of root biomass and accumulation in to the soil (Huyghe, 2003). The yield increasing and improving the quality of alfalfa is of great importance for protein problem deciding under now existing market relations.

A number of scientific studies are related to search opportunities for increasing alfalfa forage yield and seed yield by applying different agrotechnical practices – optimal density; intensity of utilization; balanced fertilization; application of foliar fertilizer, growth regulators and biostimulators (Sevov et al., 2007; Terzi et al., 2012). The yield, forage quality and the persistence of the

(*Medicago sativa* L.)

(Bouton, 2012).

(Huyghe, 2003).

(Sevov et al.,

2007; Terzi et al., 2012).

(Kores and Froud Williams, 2002; Georgieva and Nikolova, 2010; Vasileva, 2013). According to Pachev (2013) the use of liquid fertilizers contributes to faster overcoming stress cause by extreme weather conditions and increases the resistance both of plants to low temperatures and drought and the products during storage and transportation.

Sevov et al. (2007), Sevov (2011) and Vasileva (2015) found that the application of growth regulators had a positive effect on alfalfa, pea and vetch leafness. Vasileva and Kertikov, (2001; 2004) report d increasing of the seeds yield, the germination energy and germination of the seeds in sainfoin (*Onobrychis Adans.*) after treatment with biologically active substances. The growth regulators and biostimulators, as complex products containing all necessary nutrients plus humic acids and enzymes, contribute to increase the rate of photosynthesis, enhance metabolism and redistribute nutrients in the plant organism (Sabev and Pachev, 2008; Popov and Dzimotudis, 2007; Kertikov et al. (2016)

(Popov and Dzimotudis, 2007; Sabev and Pachev, 2008). Kertikov et al. (2016)

(Churkova and Lingorski, 2010; Bozhanska et al., 2017a; Bozhanska et al. 2017b, Yakimov and Ivanov, 2017, Zhekova et al., 2017).

stand are traits, in great extent determined by ensurance all of nutrients in optimal amounts (Kores and Froud Williams, 2002; Georgieva and Nikolova, 2010; Vasileva, 2013). According to Pachev (2013) the use of liquid fertilizers contributes to faster overcoming stress cause by extreme weather conditions and increases the resistance both of plants to low temperatures and drought and the products during storage and transportation.

Sevov et al. (2007), Sevov (2011) and Vasileva (2015) found that the application of growth regulators had a positive effect on alfalfa, pea and vetch leafness. Vasileva and Kertikov (2001; 2004) report d increasing of the seeds yield, the germination energy and germination of the seeds in sainfoin (*Onobrychis Adans.*) after treatment with biologically active substances. The growth regulators and biostimulators, as complex products containing all necessary nutrients plus humic acids and enzymes, contribute to increase the rate of photosynthesis, enhance metabolism and redistribute nutrients in the plant organism (Sabev and Pachev, 2008; Popov and Dzimotudis, 2007). In a study, Kertikov et al. (2016) found that regardless of the conditions (factors) and the inter-row spacing of alfalfa growing, by the highest both general and on structural elements (leaves, stems and roots) nitrate reductase activity of plants and total content of plastid pigments the biological stands treated with bio product Ecofil P were distinguished.

Results for the stimulating effect of biological products on biological, morphological and economic traits have been reported by other authors (Churkova and Lingorski, 2010; Bozhanska et al., 2017a; Bozhanska et al., 2017b; Yakimov and Ivanov, 2017; Zhekova et al., 2017).

The aim of the present study was to

Total Care
 5.
 2017
 2014-
 5.
 5 m²
 (a)
 Total Care
 200 ml da⁻¹.
 10-15 cm
 Total Care
 Lebosol,
 (N) 120 g/l; 0,9%
 (P₂O₅) 10 g/l; 2,7%
 g/l.
 () (1 g /l B); 0,3%
 (Cu) 1,5%
 (20 g/l Mn); 0,5%
 (Zn)
 (6 g/l Zn); 1,7%
 (20 g/l MgO); 11,6%
 da⁻¹
 2,5 kg
 12,5 m.
 cm,
 5
 m²,
 0,250 m²

determine the effect of Total Care foliar fertilizer application on the main morphological traits – plant height, grass stand density and on the forage productivity in Prista 5 alfalfa variety.

MATERIAL AND METHODS

The study was carried out without irrigation from 2014 to 2017 at the Experimental field of the Institute of Agriculture and Seed Science "Obraztsov Chiflik" - Rousse with Prista 5 alfalfa variety. The experimental design was a randomized block with four replications. The harvesting plot size was 10 m². The field trial included variant with Total Care product application, at 200 ml da⁻¹ application rate per treatment and control (untreated variant). Foliar fertilization was applied twice in each regrowth – at plant height of 10-15 cm and in bud stage. Total Care is an organic mineral foliar fertilizer, from Lebosol. The product containing 9,4 % Total nitrogen (N) 120 g/l; 0,9 % Total phosphorus (P₂O₅) 10 g/l; 2,7 % Total potassium oxide (K₂O) 35 g/l. Also contains: 0,05 % Water-soluble boron as boron ethanolamine (B) (1 g/l B); 0,3 % Water-soluble copper as copper nitrate (Cu) (4 g/l Cu); 1,5 % Water-soluble manganese as manganese nitrate (Mn) (20 g/l Mn); 0,5 % Water-soluble zinc as zinc nitrate (Zn) (6 g/l Zn); 1,7 % water-soluble magnesium (20 g/l MgO); 11,6 % Organic substance.

The alfalfa was sown in the optimum agrotechnical period, with 2,5 kg da⁻¹ sowing rate and 12,5 cm inter-row space. The grass stand height in cm was recorded as the majority of normally developed stems were measured from the surface of the soil to the top. The indicator was recorded in early flowering stage before every cutting. It was done in 5 places in each harvesting plot for both variants. The grass stand density, expressed by stem number per m², before cutting in each harvesting plot for each variant by sampling plot with area 0,250

(50 50 cm)

(kg da⁻¹)

105 (200 g)

kg da⁻¹.

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(ANOVA),
e a

multiple range test. Duncan's
SPSS Statistics 19.

m2 (50 cm x 50 cm) was accounted.

The forage yields (kg da⁻¹) were determined by regrowth, years and average for the study period in early flowering stage by weighing the green biomass. The dry matter content was calculated by drying the green mass sample (200 g) to constant weight in a drying chamber at 105°C. Data for green mass yield and dry matter content to dry matter yield determination (kg da⁻¹) were used.

The first and third experimental years 2 cuttings were made, and in the second and fourth years 4 cuttings.

The data for the studied traits by the One-way analysis of variance (ANOVA) method were analysed. Significance of differences was tested by Duncan's multiple range test (DMRT). The STATGRAPHICS PLUS software product was used.

RESULTS AND DISCUSSION

Regarding the meteorological conditions for the study period, determining the variation of the yield and its structural components, significant differences in both temperature sums and the amount of rainfall and its distribution by months and years were observed during the alfalfa regrowths. The year of alfalfa sowing was characterized as favourable for the development of new established stands (Table 1).

(1).

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Table 1. Meteorological characteristic of the study period

Months, decades, periods	/ Rainfall, mm					Σ t °				
	2014	2015	2016	2017	1896 -2005	2014	2015	2016	2017	1896 -2005
Σ - Σ October – March	311,6	395,7	327,2	259,7	235,1	946,7	873,4	1191,2	758,9	
/ April	7,5	21,8	22,2	2,2	14,8	109,8	72,2	156,2	109,3	99,5
	53,2	11,9	36,3	50,3	20,6	96,6	137,6	158,3	107,0	110,2
	4,1	3,5	18,1	22,7	15,6	143,9	132,5	123,0	129,1	132,2
y	64,8	37,2	76,6	75,2	50,7	350,3	342,3	437,5	345,4	341,8
/ May	19,5	5,1	40,7	43,0	16,8	138,0	175,3	127,6	152,5	152,2
	38,2	0	2,0	13,8	21,3	156,6	186,3	156,0	165,7	167,7
	09,0	14,3	55,6	33,5	28,1	213,1	207,7	209,6	188,0	192,3
y	166,7	19,4	98,3	90,3	66,1	507,7	569,3	493,2	506,2	512,2
/ June	10,7	12,9	42,9	36,1	24,1	192,4	204,5	182,1	200,3	192,3
	55,8	10,8	28,0	24,3	30,0	194,8	219,9	230,8	203,3	202,2
	12,9	41,4	3,3	0,8	26,4	197,5	190,3	247,8	251,6	212,1
y	79,4	65,1	74,2	62,1	80,5	584,7	614,7	660,7	655,2	606,6
/ July	28,2	11,8	2,2	56,8	25,0	220,2	228,5	235,0	235,2	220,1
	20,7	5,7	0,0	8,8	24,1	218,7	239,8	241,0	224,4	225,4
	18,4	1,3	0,0	19,1	18,3	262,7	294,5	286,3	267,8	252,2
y	67,3	18,8	2,2	84,7	67,4	701,6	762,8	762,3	727,4	697,7
/ August	0,1	87,4	0,0	0,0	15,8	241,6	241,3	256,7	265,9	281,0
	2,4	47,6	37,1	3,6	16,3	244,6	239,8	211,3	247,4	223,5
	32,7	69,7	17,0	30,4	17,3	243,0	234,6	240,8	222,6	235,4
y	35,2	204,7	54,1	34,0	49,4	729,2	715,7	708,8	735,9	739,8
/ September	25,2	0	0,0	41,2	14,3	204,9	223,8	229,4	209,5	193,6
	0,7	64,6	11,9	0,0	15,2	188,5	187,6	210,2	232,0	179,4
	41,2	48,6	4,8	1,0	15,1	143,6	161,2	143,4	144,5	162,3
y	67,1	113,2	16,7	42,2	44,6	537,0	572,6	583,0	586,0	535,3
Σ - Σ April - September	480,5	458,4	321,1	388,5	367,5	3410,5	3577,4	3556,1	3507,6	3426,2

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The sufficient soil moisture and high temperatures in March ensured good germination and stand density. The amount of precipitation and the temperature sums for the period April-August 2014 were close to the long-term norms and three cuts were obtained.

In 2015, the favourable soil humidity and temperature sums around the norm ensured a normal development of a first regrowth and good initial growth of plants in a second one. During next third and fourth regrowth development a long drought was occurred (except in August), which did not allow the alfalfa variety to completely manifest their potential for the studied traits. Four cuts were harvested, but unfavorable conditions affected forage yield.

The third year was characterized with prolonged rainy periods in all months of the alfalfa growing season. It led to delay of growth and development of the

2017
a,
T tal Care
5,
(2).

- alfalfa grass stands at the regrowths and three cuts were obtained.

- The meteorological conditions in 2017 were favourable with amount of rainfall and temperatures close to the average long-term norms and allowing four regrowths to be formed.

- Plant height is a variety trait which phenotypic expression is changed by the influence of internal and external factors. During studied period differences in the effect of Total Care application on the grass stand height in Prista 5 alfalfa variety, both in years and regrowths were observed (Table 2).

2. T tal Care 5

Table 2. Effect of T tal Care on grass stand height in Prista 5 alfalfa variety

Variants	/ Real plant height, m				
	2014				
	I cut	II cut	III cut	IV cut	/ Mean
TOTAL CARE	87	63	33	-	61,00 a
	75	55	30	-	53,33 b
(+/-) / Diference (+/-)	+12	+8	+3	-	+7,67
LSD 0,05					3,84
	2015				
TOTAL CARE	92	62	51	57	65,50 a
	103	53	42	54	63,00 b
(+/-) / Diference (+/-)	-11	+9	+9	+3	+3,50
LSD 0,05					2,21
	2016				
TOTAL CARE	63	66	51	-	60,00 a
	58	59	51	-	56,00 b
(+/-) / Diference (+/-)	+6	+7	-	-	+4
LSD 0,05					3,24
	2017				
TOTAL CARE	106	83	85	20	73,50 a
	97	87	82	17	70,75 a
(+/-) / Diference (+/-)	+9	-4	+3	+3	+2,75
SE					2,95

LSD 95% -

LSD 95% - values in the columns followed by the same letter are not significantly different

tal Care

- The results obtained showed that Total Care application had a strong effect on the grass stand height in the first regrowth for the first alfalfa growing

12 m.

3 m.

7,67 m

Care

9 m.

3,5 m.

m.

3 m²,

63 m 58

tal Care

season, exceeding the control by 12 cm. In the second and third regrowths, the trend for positive influence of the product on the studied trait was kept. Treated stands were higher than the control 8 m 3 m, respectively. It was also found that stimulate affect of the product was decr ased at each following regrowth. In the first productive year higher plants for stands with Total Care application were established significantly exceeding the control by 7,67 m.

A different effect of the product on the trait was observed during the second growing season. The data showed the strongest positive impact of Total Care in the second and third regrowth, with a reported excess of 9 cm. Average for the second harvest year the height of treated grass stand exceeded significantly the height of untreated stand with 3,50 cm.

The reported values for the trait in first cut of the third growing season were 63 cm and 58 cm in the treated and untreated variants, respectively. In a second regrowth the trend for positive influence of the Total Care on the trait was kept, and in third one the stands height for the both variants (with and without leaf fertilization) was equal.

It was observed a stronger phenotypic expression of the genes controlling the trait at the treated stands in the fourth year, but the positive influence of the product was not significant.

The results for the grass stand density trait, expressed by stems number per m² are presented in Table 3. The trait values showed that in the first regrowth in the alfalfa establish year the difference between a treated variant and the control was not significant.

3. T tal Care

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Table 3. Effect of T tal Care on grass stand density in Prista 5 alfalfa variety

Variants	m ² / Stem number per m ²				
	2014				
	I cut	II cut	III cut	IV cut	Mean
TOTAL CARE	85	136	66	-	95,66 a
/Control	83	127	58	-	89,33 b
(+/-) / Diference (+/-)	+2	+9	+8	-	+7,09
LSD 0,05					4,69
	2015				
TOTAL CARE	490	452	446	417	451,25 a
/Control	464	394	430	404	409,33
(+/-) / Diference (+/-)	+24	+58	+26	+13	+41,98
LSD 0,05					40,09
	2016				
TOTAL CARE	1172	454	440	-	688,00 a
/Control	1192	496	484	-	724,00 a
(+/-) / Diference (+/-)	-20	-42	-44	-	-36
LSD 0,05					72,32
	2017				
TOTAL CARE	537	439	346	146	367,00 a
/Control	533	409	356	121	354,75 a
(+/-) / Diference (+/-)	+4	+9	+10	+25	+12,25
SE					57,88

LSD 95% -

LSD 95% - values followed by the same letter are not significantly different

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A stronger stimulating effect of T tal Care on the stems productivity in the second and third cuts was found. The mean values reported indicated that during the first experimental year Toltal Care was contributed to the development of significantly better grass stand density.

The data showed positive response of Prista 5 variety to the treatment with the organic mineral foliar fertilizer, as forming more stems per unit area (490) compared to the control (464) in first cut at the second year, which was characterized by a prolonged period of drought. The variant including Toltal Care application was again shown a significantly better potential for stems formation in a second cut. There were established a decreases in the stimulating effect of the product in the following third and fourth cuts. The average trait values indicated that the

	5				
		41,98	/m ²		
Care				T tal Care	
				a	
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				T tal Care	
Care					
864,7 kg da ⁻² ,					
(745,9 kg da ⁻²)					15,91%.
10,87%					
5,38%					

- treated grass stand of Prista 5 variety had
- a better stem productivity per unit area
- during the second harvest year, and the
- increase of 41,98 stems/m² compared to
- the control was significant. It can be
- noted that in more dry years the product
- has a clearer expressed positive impact
- on stem formation.

- he results indicated that during
- the 2016 T tal Care was not contributed
- to the development more stems per unit
- area. During the fourth growing season,
- the strongest stimulating effect of Total
- Care in the fourth regrowth was found,
- when for the both variants the genes
- controlling the stems formation was
- distinguished with the lowest phenotypic
- expression. It was found that in the last
- alfalfa productive year the treated variant
- had an equal grass stand density with the
- control.

- The formation of alfalfa forage yield
- is a complicated process due to the
- interaction of plants with agroclimatic and
- soil conditions.

- Data for dry matter yields are
- presented in Table 4. There were
- observed significant differences in the
- effect of Total Care application on the
- trait in Prista 5 alfalfa variety, both in
- regrowths and in years.

- In the first regrowth during first
- growing season, for the stand treated
- with Total Care 864,7 kg da⁻² dry matter
- yield was reported, exceeding with
- 15,91% the yield for the control (745,9 kg
- da⁻²). The values reported indicated, that
- the product application contributed to
- higher yields compared to the control in
- the following two cuts by 10,87% and
- 5,38%, respectively. Similarity to the
- grass height data decreasing of the
- stimulating impact of the foliar fertilization
- on the yield at each subsequent regrowth
- was established.

4. T tal Care

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Table 4. Effect of T tal Care on forage productivity in Prista 5 alfalfa variety

Variants	, kg da ⁻² / Dry matter yield, kg da ⁻²				
	2014				
	I regrowth	II regrowth	III regrowth	IV regrowth	/ Total
TOTAL CARE	427,2	350,4	87,1	-	864,7 a
/Control	347,3	316,0	82,6	-	745,9 b
%	23,00	10,87	5,38	-	15,91
LSD 0,05					60,23
	2015				
TOTAL CARE	570,4	522,1	208,5	80,4	1381,4 a
/Control	550,0	437,5	180,6	73,5	1241,5 b
%	3,71	19,35	15,48	9,39	11,27
LSD 0,05					78,65
	2016				
TOTAL CARE	1056,0	368,0	341,8	-	1765,8 a
/Control	989,0	422,4	303,6	-	1715,0 a
%	6,77	-12,88	12,58	-	2,96
LSD 0,05					122,40
	2017				
TOTAL CARE	738,4	392,6	648,1	148,5	1927,6 a
/Control	639,4	390,0	587,4	172,8	1789,6 a
%	15,49	0,67	10,33	-14,06	7,71
SE					144

LSD 95% -

LSD 95% - values followed by the same letter are not significantly different

Care, 570,4 kg da⁻²
550 kg da⁻²
tal Care
19,35%; 15,48%
9,39%.
tal Care
5.
tal Care

Data showed positive reaction of alfalfa to treatment with Total Care in the first cut, during the second growing season. For both treated variant and control the dry matter yield obtained were 570,4 kg da⁻² and 550 kg da⁻², respectively. A significant stimulating effect of Total Care on the phenotypic expression of the trait in the next three cuts was found. The foliar fertilization, was led to an increase in dry matter yield with 19,35%; 15,48% 9,39%, respectively compared to the control. The summarized data for the first and second year of the study showed a strong positive impact of Total Care on the total annual dry matter yield from grass stands of Prista 5 variety. The differences between values reported for both variants were significant.

The trend of significant differences in the effect of treatment with Total Care in each regrowths was kept during the

third and fourth growing seasons of the crop. The product application had a positive effect on the trait phenotypic expression only in first cut of the third productive year and in the first and third regrowths of the fourth growing season. The summarized results showed that the treatment with Total Care was contributed to the higher dry matter yields in the both years, but the excesses compared to the control were not significant.

It was also established that the degree of positive impact of the product decreases at each subsequent growing season.

The comprehensive evaluation of data in respect the plants height morphological trait showed that for the study period under the soil and meteorological conditions of IASS "Obraztsov chiflik", the treated whit Total Care alfalfa stand significantly exceeded the control by 8,15% (Table 5). There was observed a slight stimulating effect of the foliar fertilization on the grass stand density, expressed by stems number per unit area.

5, 8,15% (5). (m²).

5. Total Care a

5, 2014-2017 .

Table 5. Effect of Total Care on grass stand height, grass stand density and forage productivity in Prista 5 alfalfa variety from 2014 to 2017

Variants	2014-2017		
	Mean grass stand height, m	Mean stem number per m ²	Mean dry matter yield, kg da ⁻²
Total Care	65,09 a	403,25 a	1484,88 a
/ Control	60,62 b	398,20 a	1373,00 b
%	7,37	1,27	8,15
SE	2,10	36,01	72,65

LSD 95% -

LSD 95% - values in the columns followed by the same letter are not significantly different

5, 8,15% - Total Care -

The mean annual dry matter yield at treated grass stand of Prista 5 variety with the Total Care foliar fertilizer was 8,15% higher than the control, as the difference was significant.

The stimulating impact of the

a - product on dry matter yield decreased in each subsequent growing season.

CONCLUSIONS

The summarized results both by regrowths and years showed a different effect of Total Care organic-mineral foliar fertilizer on the morphological traits studied and on the forage productivity in Prista 5 alfalfa variety, in response to the changes on a given factor of environment.

Total Care product stimulated the development of significantly higher alfalfa grass stand and had a slight positive impact on the potential for stem formation.

It had a clearly expressed positive effect on dry matter yield, as the mean annual yield at a treatment was by 8,15% higher than the this one for the control.

The stimulating effect of Total Care on the productive potential of variety decreased in each subsequent growing season.

The obtained results give a reason to consider that the phenotypic expression of the morphological traits – plants height and stem number and of the forage productivity genetic potential is a result of the complex relationship genotype x foliar fertilization x environment conditions.

Total Care

5,

Total Care

8,15%

Total Care

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In situ ex situ

***Juniperus sibirica*
Burgsd. (Cupressaceae)**

” ” 24, 4000 ,

**In situ and ex situ study on the possibilities
for generative propagation of *Juniperus sibirica*
Burgsd. (Cupressaceae)**

Tzenka Radoukova

University of Plovdiv “Paisii Hilendarski”, Faculty of Biology, Department of Botany and
Methods of Biology Teaching, 24 “Tzar Assen” Str., Plovdiv 4000, Bulgaria

E-mail: kiprei@abv.bg

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SUMMARY

Juniperus sibirica Burgsd.
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3
EX SITU
IN SITU:

The effect of some factors on the possibilities for generative propagation of *Juniperus sibirica* Burgsd. under controlled laboratory and natural habitat conditions of the species was studied. Green 3-year old galbuli (cones) and mature galbuli, as well as their seeds, were used to perform the analyses. The results obtained show that galbulus maturity is of greatest importance for germination. The period of germination in the seeds of the green 3-year old galbuli was significantly shorter, but the number of the obtained seedlings was smaller. Concerning the other factors studied under EX SITU conditions, the most important effect on germination was exerted by the time of sowing the galbuli and seeds, as well as the temperature and humidity of the environment, and, under IN SITU conditions: the removal of the fleshy coat and the presence or absence of soil cover

Burgsd., *Juniperus sibirica*

over the generative organs.

Key words: generative propagation, *Juniperus sibirica* Burgsd., galbuli, laboratory conditions, natural conditions

INTRODUCTION

(1500 - . . .)
Juniperus sibirica Burgsd. (Yordanov, 1963-1967).

In the recent decades, a mass distribution of the ground spreading, strongly diverging perennial evergreen shrub was observed in the highland forest-free zone of Bulgarian mountains (above 1500 m asl), which in 'Flora of Bulgaria' is defined as *Juniperus sibirica* Burgsd. (Yordanov, 1963-1967).

Stefanov (1943)

The high mountainous forest-free zone of Troyan Balkan is one of the typical areas of wide distribution of Siberian juniper.

(2000, 2001)

Meshinev

J. sibirica

Different reasons for the infestation of Siberian juniper were found in the different studies. According to Stefanov (1943) this is due to the artificial destruction of forest vegetation in the mountains. Studies of Meshinev (2000, 2001) defined a part of *J. sibirica* populations in Bulgaria to be of primary origin in the natural plant cover – mainly around the upper forest border and in the open areas not occupied by *Pinus mugho* Poir. In cases of logging or burning of creeping pines, *J. sibirica* gradually spreads as an alohtone assectator and develops in the new environmental conditions as a degressive dominant and edificator (Bondev, 1991). A number of authors associated the increase of the territories occupied by the species with the greatly reduced grazing regime due to the economic changes in Bulgaria over the last few decades (Totev, 1973; Velev and Apostolova, 2008; Pedashenko et al. 2015).

Pinus mugho Poir.

, *J. sibirica*

(Bondev, 1991).

(Totev, 1973; Velev and Apostolova, 2008; Pedashenko et al., 2015).

J. sibirica,

Despite the significant enlargement of the areas occupied by *J. sibirica*, the concrete data on the biology of the species and especially the ways of its reproduction and distribution under the

				- natural habitat conditions are scarce.
				- The natural propagation of juniper by seeds depended on a number of biological and ecological factors: sexual distribution of plants, pollen vitality, the amount of galbuli and seed quality, pollination and fertilization conditions, seed germination (Garcia et al., 2000). According to Chambers et al. (1999) the distribution agents and the seedling viability rate were also of great importance.
2000). Chambers et al. (1999)				
	15			
<i>communis</i> L.			J.	After a 15-month artificial stratification of <i>J. communis</i> L. seeds in the northwestern parts of Scotland, Broome (2003) reported a mass seedling emergence by the fifth year, the most active seedling growth being reported after 2 years and 7 months (80% of the total number of germinated seeds).
		, Broome (2003)	5	
	2	7	(80%	
)				
		, Zheronkina (1971)	-	In order to shorten the period of seed germination and emergence by about a year, Zheronkina (1971) carried out an experiment using special equipment, with unripe seeds of common and Virginia juniper, which had not entered a period of deep dormancy yet. The galbuli were collected in September – October, selecting them not to be dark in colour. Up to 90% of <i>J. communis</i> and 41% of <i>J. virginiana</i> L. seedlings emerged as early as the next spring.
<i>communis</i>	41%	90%	J.	The analysis of the age structure of <i>J. sibirica</i> communities on the same territory showed the existence of young seedling plants, suggesting the possible generative reproduction of the species in its natural habitat (Radoukova, 2011). The relatively high percentage of formed galbuli could be the possible prerequisite. Kachaunova (2001) defined the share of the galbuli to be 0.14% of the total phytomass of <i>J. sibirica</i> in Pirin Mountain, Bulgaria. The assessment of Evstatieva (2014) about the potential annual yield of mature blue galbuli in sample plants of <i>J. communis</i> in the region of Central Balkan
<i>J. sibirica</i>				
"		"		
(Radoukova, 2011).				
Kachaunova, (2001)			0.14%	
<i>sibirica</i>			J.	
		Evstatieva, (2014)		
		<i>J. communis</i>		

680 .
 1-3 .
 (Diotte and Bergeron, 1989; Ivanova, 1982).
 (Zheronkina, 1971; Broome, 2003).

(in situ) (ex situ)

mountain (Bulgaria) showed 680 grams of galbuli per plant. Despite the relatively large amount of galbuli, generative reproduction of juniper is limited by the 1-3 years long period of deep seed dormancy (Diotte and Bergeron, 1989; Ivanova, 1982). Overcoming it is a precondition for carrying out further studies on the processes of germination and sprouting in artificial environmental conditions (Zheronkina, 1971; Broome, 2003).

The aim of the present study was to analyze the impact of some factors on the possibilities for generative propagation of Siberian juniper under natural (in situ) and artificial (ex situ), conditions as one of the prerequisites for mass distribution of the species in the highland areas of Bulgaria.

MATERIAL AND METHODS

J. sibirica
 – 2013 . 2015 . () 2017 . ().
 Zheronkina (1971) Pack (1921), 2013 .
 3 , Zheronkina (1974), Kozhevnikova (1986). 2015 .

The experiment on studying the capacity of *J. sibirica* for generative propagation was carried out at three stages – in 2013 and 2015 (under field conditions) and in 2017 (under laboratory conditions). The experimental variants were set, following the methodological guidelines of Pack (1921), Zheronkina (1971) and others. In 2013, whole fully ripe blue galbuli were used for the experiment in the natural habitat, as well as 3-year-old green galbuli, because according to Zeronkina (1974) and Kozhevnikova (1986) they had a fully mature embryo. In 2015 the experiment was carried out with the seeds from the two variants of the galbuli, which fleshy coat was removed. The same variants of galbuli and seeds were set under laboratory conditions, at controlled temperature and humidity.

(IN SITU)

The galbuli were planted in the natural habitat (IN SITU) on the territory of Beklemeto area, part of the Central Balkan National Park, where the Siberian juniper manifests its obvious capacity to occupy new territories. 50x50 cm plots

« , »,

50 50
 cm 5 :
 (15.03.2013 .);
 11.03.2015 .);
 (1.05.2013 .; 5.05.2015 .);
 (21.06.2013 .);
 22.06. 2015 .);
 (4.09.2013 .; 10.09.2015 .);
 (18.10.2013 .; 23.10.2015 .).
 1 m
 50 . ; 50 .
 (2013 .); 150
 . 150 .
 (2015 .).
J. sibirica
 .(Tabaeva, 1994).
 3
 2
 -
 (EX SITU)
 1 min
 1% KMnO₄
 .
 600 .
 (3-)
 1800 .
 (300 .)
 t= - 20⁰ .

were used at 5 stages: until the beginning of the mother plant growth (March 15, 2013; March 11, 2015); beginning of vegetation (May 1, 2013; May 5, 2015); beginning of an intensive growth (June 21, 2013; June 22, 2015); slowing down of the intensive growth (September 4, 2013; September 10, 2015); after the end of the active vegetation (October 18, 2013; October 23, 2015). The variants of planting the galbuli were over a bare soil surface without a natural grass cover and at a depth of 1 cm in the soil. The following galbuli were used: 50 mature galbuli; 50 green three-year-old galbuli (in 2013); 150 seeds of mature galbuli and 150 seeds from green three-year-old galbuli (in 2015). The number of seeds to be used was decided considering the fact that one galbulus of *J. sibirica* usually contains 3 seeds (Tabaeva, 1994). Based on this dependence, the percent of the seed plants obtained was also calculated.

As the aim of the study was to keep close to the natural conditions of the species development, the galbuli and seeds were not subject to pre-treatment. Observations were performed twice during the vegetation – in spring and autumn. When reporting the results, a major attention was paid to the emergence of seedlings, as eventual digging of the soil cover could cause mechanical damages to the planted galbuli and seeds.

Under laboratory conditions (EX SITU), the galbuli and cleaned seeds were pre-washed for about 1 min in 1% KMnO₄ solution and placed on a filter paper in disinfected Petri dishes. The seeds were extracted from the galbuli manually.

At each stage of the initial planting 600 galbuli were used (3-year-old green and mature) and 1800 seeds. Half of the galbuli (300) from each stage of ripeness were placed at t = -20° C. Every 30, 60,

30, 60, 90 100 .

t= 0 +5° .

30

25 .

t= +10 +15° . . .

25 30, 60 90 .

0+5° .

25 .

t= 0+5°

300 .

t= 0 + 5° ,

30 50 .

t= +10 +15° . .

30, 60, 90, 120 150 .

50 .

4 . . .

779

(1).

2016 .,

(04.09.2013 .),

– 1120

3 .

5.

8 (1).

90 days, 100 galbuli were taken out from the freezer and stored at t of 0 to 5° C. After that, every 30 days 25 galbuli were selected from each hundred and placed at t of 10 to 15° C. i.e. every batch of 25 galbuli were kept at t of 0 to 5° C for a period of 30, 60 to 90 days. The last 25 galbuli were left at t = 0 + 5° C until eventual germination.

The other 300 galbuli were placed at t = 0 + 5° C and at every 30-day interval 50 galbuli were shifted at t = +10 +15° C, i.e. they were left at low temperatures for 30, 60, 90, 120 and 150 days, respectively. The remaining 50 galbuli were left at the initial temperature.

The planting scheme of the seeds was identical to that of the galbuli but their amount was tripled to match the number of seeds in the planted galbuli. In all the studied variants, humidity was maintained by spraying with distilled water once a week.

RESULTS AND DISCUSSION

Planting galbuli and seeds under natural conditions (in situ).

The emergence of seedlings from the green 3-year-old galbuli planted on September 4, 2013 at a depth of 1 cm in the soil, was recorded at the end of 2015 (4 seedlings), i.e. the period of seedling emergence was about 779 days or approximately 2 years (Table 1).

In 2016 emergence of seedlings was recorded from galbuli planted at the same time (September 4, 2013), but in mature galbuli the period of seedling emergence was 1120 days or approximately 3 years. In the variant with putting the galbuli on the soil surface, the number of seedling plants obtained was 5. In the variant with planting the galbuli beneath the soil surface, the number of seedlings was 8 (Table 1).

2015 . ,
 2016 . -
 1 . -
 (10.09),
 2013 .,
 (385).
 2017 . ,
 10.09. 2015 . 3
 10 . 1
 cm .
 2015 ., 23.10.
 - 2 . (1).
 , -
 Zheronkina
 (1971),
J. communis L. *J. virginiana*
 L.
 Kozhevnikova
 (1986)
 (*J. sibirica*)
 -
 -
 (Zheronkina, 1974).
 ,
 ,
 (1 cm)

The earliest emergence of seedlings from the seeds planted in 2015 was reported at the end of 2016 for the seeds from green three-year-old galbuli – one seedling in each of the two variants with and without a soil cover. The seedlings were planted in the period of slowing down of the intensive growth of the mother plants (10 September), which corresponded to the results obtained in 2013, but the period of emergence was shortened by about a year (385 days). At the beginning of 2017, seedlings obtained from mature galbuli were also reported: from those planted on September 10, 2015, they were 3 in number in the variant without a soil cover and 10 in the variant planted at the depth of 1 cm in the soil. For the same period, plants also emerged from the seeds of the mature galbuli planted on October 23, 2015 – one seedling was obtained from the variant with putting the galbuli on the surface and 2 seedlings in the variant with a soil cover (Table 1). The result obtained confirmed the tendency for the slower germination of the seeds from mature galbuli. Similar data were mentioned by some authors in experiments for artificial propagation of juniper species. According to Zheronkina (1971), planting of unripe seeds of *J. communis* L. and *J. virginiana* L. accelerated seedling emergence by one year and shortened the growth period of the propagating material. Kozhevnikova (1986) also noted that immature green galbuli with morphologically mature seeds can be used to accelerate the germination process in juniper species (*J. sibirica* being among them). The reason for the slower germination of the mature galbuli was that when the galbuli reached anatomical and morphological maturity, the seeds entered a state of deep dormancy (Zheronkina, 1974). The results obtained in the present study showed that after that period, the number of germinated seeds increased. Under the same planting conditions (1 cm under the soil surface), mature galbuli produced

6 (1%),
29 (4.8%)

2).

- more seedling plants than green galbuli. The seedlings obtained from all the green three-year-old galbuli and seeds from them in the natural habitat of the species were 6 (1% of the total amount of seeds), while from the mature galbuli and seeds from them the obtained plants were 29 (4.8% of the total amount of seeds), (Table 2).

1. (ex situ) (in situ)

Table 1. Results obtained from natural (in situ) and laboratory (ex situ) conditions

Type of placed generative organs	Period of planting	Conditions of planting	Degree of maturity	Reporting of Seed Plants		
				Date	Number	() Duration (days)
(in situ)						
Planting galbuli and seeds under natural conditions (in situ)						
Galbuli	04.05.2013	1 cm With 1 cm soil cover	3 . 3 y. green	23.10.2015 .	4	779
			Mature	30.09.2016 .	5	1120
		1 cm With 1 cm soil cover			8	1120
Seeds	10.09.2015	Over a bare soil surface	3 . 3 y. green	30.09.2016 .	1	385
		1 cm With 1 cm soil cover			1	385
		Over a bare soil surface	Mature	12.05.2016	3	609
	1 cm With 1 cm soil cover	10			609	
	23.10.2015	Over a bare soil surface			1	566
		1 cm With 1 cm soil cover		2	566	
(ex situ)						
Seed germination under laboratory conditions (ex situ)						
Seeds	17.08.2015	t°C = 0 + 5	3 . 3 y. green	18.03.2017	2	214

Pack (1921) - According to Pack (1921) the germination of juniper seeds under natural conditions was very slow and the

1%. (2001) *J. communis* Garsia
 36%. Ivanova (1982)
 1-2
 Broome (2003)
 Juniperus. a
 1 cm (2). Hofmann (1917)

germination rate was only about 1%. In the high-mountainous areas of the Mediterranean region, Garsia (2001) found seedlings of *J. communis* in the second and third spring after planting, the germination rate being 36%. According to Ivanova (1982), under natural conditions, the seeds of the juniper species emerged 1-2 years after maturing and falling to the ground.

Under the conditions of Beklemeto area, the removal of the fleshy coat and planting of clear seeds shortened the period of emergence by about a year and increased, although insignificantly, the number of the seedlings produced. Broome (2003) reported a double increase in germination in different *Juniperus* species when removing the fleshy coat of the galbuli.

The mature galbuli and seeds from them, planted on the soil surface, under the conditions of Beklemeto area, formed seedlings for the same period of time as the mature galbuli covered with 1 cm of soil, but the amount of germinated seeds was significantly higher (Table 2). That determines the planting depth also as a factor influencing the rate of emergence. According to Hofmann (1917), the difficult germination of juniper seeds could be controlled by changing the depth of the soil cover.

2.

(in situ)

Table 2. Emergence of seedlings under natural conditions (in situ)

Date of the betting	Three-year-old grin galbuli				Mature galbuli			
	On the soil surface		1 cm With 1 cm soil cover		On the soil surface		1 cm With 1 cm soil cover	
	.	%*	.	%*	.	%*	.	%*
2015								
4.09.	0	0	4	0.7	5	0.8	8	1.3
2017								
10.09	1		1	0.3	3	0.5	10	1.7
23.10	0		0	0	1	0.2	2	0.3

(ex situ).

Seed germination under laboratory conditions (ex situ).

Under laboratory conditions besides the two factors: period of planting and the stage of maturity of the galbuli, the influence of temperature and humidity were also considered.

2015 . - Germination of 2 seeds collected from green three-year old galbuli on August 17, 2015 and placed at a constant temperature of 0+5°C, was reported on March 18, 2016. The duration of the process was 214 days (Figure 1).

(1).



Fig. 1. Sprouted seeds of green goblins set on 17.08.2015

17.08.2015

0.0028%.

(49.94%),

(30.22%)

(19.61%).

(3).

Under laboratory conditions, the germination rate was about 0.0028%, reported for all the seeds and galbuli included in the study.

The multifactorial analysis shows that the most significant influence on the number of appeared seeded plants is the duration of the growth period (49.94%), followed by the degree of maturity of the galbuli (30.22%) and the presence of soil cover (19.61%). The type of betting generative organs impacts less on the number of seed plants obtained, but significantly shortens the growing period (Table 3).

3.

Table 3. Degree of influence of the studied factors

	% ² Degree of influence % ²	Std. Error	Significant
Duration of growth	49.94	0.11	0.04
Degree of maturity of the underlying galbuli and seeds	30.22	0.17	0.04
Presence of ground cover	19.62	0.01	0.03
Type of bedding material (galbula/seed)	0.21	0.19	0.03

CONCLUSIONS

J. sibirica

Generative propagation of *J. sibirica* is hampered by the period of deep dormancy of the seeds. That determines the insignificant share of propagation by seeds for the mass distribution of the species in the high mountainous areas of Bulgaria.

The different stage of maturity of the galbuli has a great influence on the time of germination. Planting green galbuli and seeds from them accelerated that process by about a year, but the amount of seedlings formed was significantly less. Even a thin soil cover over the planted galbuli favour their germination.

Considering the other studied factors, the time of planting the galbuli has a strong effect on the germination process. The most favourable of the 5 stages was that at the end of the active growth of the mother plants.

Keeping the galbuli and seeds at a temperature below -20° C has a negative effect on the process of germination and sprouts were reported neither from galbuli, nor from the seeds after being exposed at that temperature.

The fleshy coat also has a similar inhibitory effect on germination. Its decomposition under laboratory conditions is impossible, which is the reason for the lack of germination in all

Juniperus

100

10

- the variants of planted galbuli. Under the conditions of the natural habitat, the fleshy coat of the galbulus delays the process of germination by about a year.

- The germination of the *Juniperus* seeds according to different studies varies from 100 days to 10 years and more, which implies the possibility of subsequent future germination of the seeds and galbuli planted in the present study.

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