

Research Regarding the Improvement of Mechanisms for Adaptation to Alternative Hydrothermal Stress of Some Species and Mixtures of Perennial Forage Grasses

Mihai Costescu*, Elena Taulescu, Andreea Andreoiu, Carmen Comănaș

Research-Development Institute for Grasslands, 500128 Brasov, Romania

*E-mail: mihai.costescu@pajisti-grassland.ro

Original scientific paper

SUMMARY

The research objective consists in establishing the optimal mixtures of perennial grasses and forage legumes for providing a grass carpet resistant to hydrothermal stress and a high nutritional value. It has made observations on the behaviour of the grass mixture plants composed in different proportions under the stationary conditions from the mountain area. The grass species studied are: *Dactylis glomerata*, *Phalaris arundinacea* and *Bromus inermis*, and the legume species is *Medicago sativa*.

The research is carrying out in the experimental fields of the Research-Development Institute for Grasslands Brasov, under specific field conditions in the south-east of Transylvania, the experiences being placed in 2018 and being in progress. The mixtures used are simple, consisting of one grass and one forage legume, in which the participation percentage of the grasses increases from 25 % to 100 %, and the proportion of the perennial forage legume representing the difference.

(*Dactylis glomerata*,
Phalaris arundinacea *Bromus inermis*)
(*Medicago sativa*)

E
2018

()

25% 100%,

Based on the chemical analysis and the data obtained in the laboratory are established optimal mixtures to provide resistance to hydrothermal stress in the experimental area, as well as from the similar areas by climate point of view.

The aim of using these simple mixtures is that to obtain fodder with high nutritional qualities and a high production, under conditions of climate change.

Key words: mixtures, grasses, legumes, hydrothermal stress, nutritional qualities, climate change

INTRODUCTION

One of the current problems with great importance worldwide, is represented by the emergence of extreme weather phenomena that present a higher frequency.

This reality also requires important changes in the strategy of the fodder production to ensure the necessary quantities of green mass and fodder in different preservation forms (wilted hay, hay, semi silage, silage, briquettes etc.), for a high performance animal breeding.

One of the main phenomena with a high risk for agriculture, is associated with the meteorological drought, a phenomenon characterized by the marked decrease of rainfalls, over a longer period and on large areas.

The main effects of the drought, depending on its starting, are the lateness or absence of germination, the delayed emergence of the plants, a reduced vegetative development, the limitation of the vegetative development in a certain period of plant vegetation stage, diminishing the multiplication of plants.

Providing the animal feed needs must be achieved by increasing the forage production area, by establishing adequate structures, by promoting in crop systems the perennial legume species in pure culture, or in mixing with perennial grasses as well as by cultivating high-yielding varieties quality and production (Moga and Schitea, 2005; Schitea, 2010).

(Moga and Schitea, 2005; Schitea, 2010).

Based on the results achieved at the Research-Development Institute for Grasslands Brasov as well as the collaborating research stations, a zoning of the main species of perennial grasses and forage legumes was taken into consideration informing the following aspects:

- the biological characteristics of the perennial species of forage grassland plants;
- their spread in spontaneous flora;
- their demands on climate and soil conditions.

MATERIAL AND METHODS

In the paper is presented an experimental field, located at Research-Development Institute for Grassland Brasov, from Romania, with grass species: *Dactylis glomerata*, *Phalaris arundinacea* and *Bromus inermis*, and the forage legume species *Medicago sativa*. The mixtures used are simple, consisting of one grass and one forage legume, where the participation percentage of the grasses increases from 25 % to 100 %, and the proportion of the perennial forage legume representing the difference.

From the geomorphological point of view, the territory of the experimentation zone is within the Eastern Carpathians Unit (I), Moldo-Transilvanian Carpathians (B), Brasov Depression (g), Bârsei Depression (1).

The territory of the Institute of Research and Development for Grasslands Brasov is climatically within the moderate continental climate sector.

According to the Köppen classification system, the experimentation zone falls within the climatic region D.f.b.x. subtype K, in which:

D = boreal climate, rainy, with cold winters and snow;

f = sufficient rainfall throughout the year;

b = average temperature of the hottest month, below 20 °C;

x = precipitation at the end of winter;

K = cold winters, dry summers, average

Based on the results achieved at the Research-Development Institute for Grasslands Brasov as well as the collaborating research stations, a zoning of the main species of perennial grasses and forage legumes was taken into consideration informing the following aspects:

- the biological characteristics of the perennial species of forage grassland plants;
- their spread in spontaneous flora;
- their demands on climate and soil conditions.

MATERIAL AND METHODS

In the paper is presented an experimental field, located at Research-Development Institute for Grassland Brasov, from Romania, with grass species: *Dactylis glomerata*, *Phalaris arundinacea* and *Bromus inermis*, and the forage legume species *Medicago sativa*. The mixtures used are simple, consisting of one grass and one forage legume, where the participation percentage of the grasses increases from 25 % to 100 %, and the proportion of the perennial forage legume representing the difference.

From the geomorphological point of view, the territory of the experimentation zone is within the Eastern Carpathians Unit (I), Moldo-Transilvanian Carpathians (B), Brasov Depression (g), Bârsei Depression (1).

The territory of the Institute of Research and Development for Grasslands Brasov is climatically within the moderate continental climate sector.

According to the Köppen classification system, the experimentation zone falls within the climatic region D.f.b.x. subtype K, in which:

D = boreal climate, rainy, with cold winters and snow;

f = sufficient rainfall throughout the year;

b = average temperature of the hottest month, below 20 °C;

x = precipitation at the end of winter;

K = cold winters, dry summers, average

temperature of the hottest month between 18 - 22 °C.

For 2018-2019 agricultural year, the lowest monthly average temperatures were recorded in December 2018 with the value of -1.27 °C, with a positive deviation of 0.4 °C from the multiannual average of -1.7 °C and in January 2019 this value was -2.37 °C with a positive deviation of 1.5 °C compared to the multiannual average of the month which was -3.9 °C (Table 1).

temperature of the hottest month between 18 - 22 °C.

For 2018-2019 agricultural year, the lowest monthly average temperatures were recorded in December 2018 with the value of -1.27 °C, with a positive deviation of 0.4 °C from the multiannual average of -1.7 °C and in January 2019 this value was -2.37 °C with a positive deviation of 1.5 °C compared to the multiannual average of the month which was -3.9 °C (Table 1).

1. (°C) 2018-2019, ICDP
Table 1. Temperature values (°C) for the agricultural year 2018-2019, at ICDP Brasov

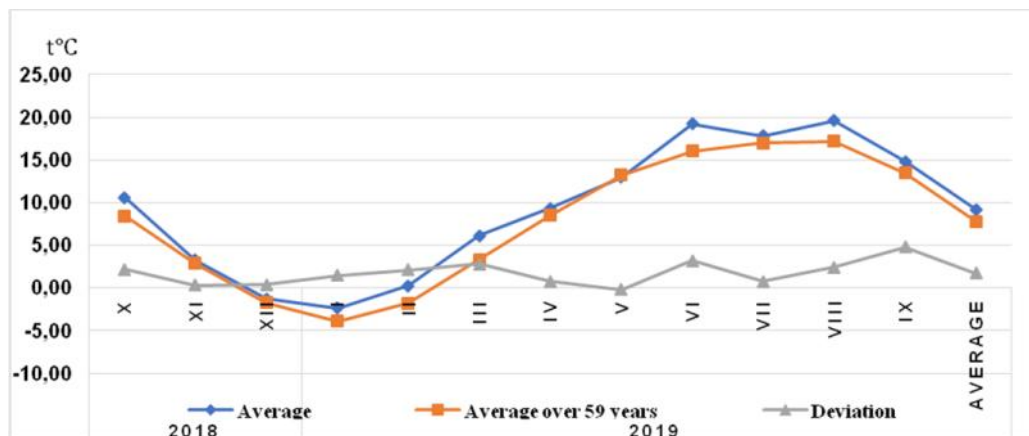
/Year	2018			2019									/Average
/Month	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX	/Average
/Average	10.6	3.23	-1.27	-2.37	0.27	6.13	9.3	13.0	19.2	17.8	19.57	14.8	9.18
⁵⁹ Average over 59 years	8.4	2.90	-1.70	-3.90	-1.80	3.30	8.5	13.2	16.0	17.0	17.20	13.5	7.80
/Deviation	2.2	0.30	0.40	1.50	2.10	2.80	0.8	-0.2	3.2	0.8	2.40	4.8	1.70

14.0 °C, 5.5 °C

15.9 °C, 17.8 °C 18.5 °C, 2.7 °C, 1,8 °C 1,5 °C

(1).

In April, an average temperature of 14.0 °C was recorded, with a positive deviation of 5.5 °C compared to the average annual temperature of the month. Also, in May, June and July, the average temperature recorded was 15.9 °C, 17.8 °C and 18.5 °C respectively with positive deviations of 2.7 °C, 1.8 °C and 1.5 °C respectively compared with the multiannual averages of the respective months (Figure 1).



1. 2018-2019
Fig. 1. Temperature evolution during 2018-2019 agricultural year

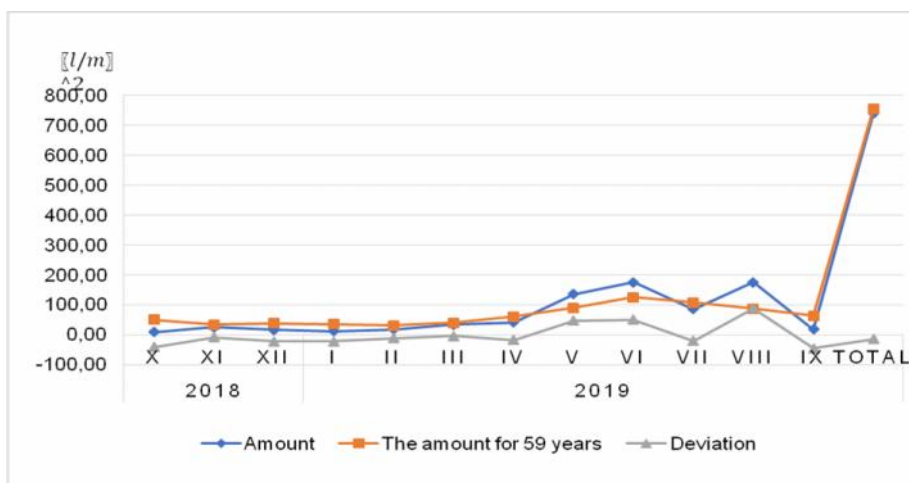
Precipitation recorded negative deviations for most of the year compared to the multiannual average. The total amount of rainfall recorded was 737.2 l/m² (Table 2), with a negative deviation of -16.0 l/m² compared to the multiannual average recorded over a 59 years period which was 753.2 l/m².

2. (l/m²) 2018-2019, ICDP
Table 2. Rainfall values (l/m²) for the agricultural year 2018-2019, at ICDP Brasov

/Year	2018			2019									Total
/Month	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX	Total
/Amount	8.6	24.2	15.1	11.8	16.7	33.4	41.2	134.4	174.9	85.3	174.6	17.0	737.2
59 Amount for 59 years	50.0	33.4	37.9	34.7	29.6	38.5	59.0	88.5	124.8	107.2	86.9	62.7	753.2
Deviation	-41.4	-9.2	-22.8	-22.9	-12.9	-5.0	-17.8	45.9	50.1	-21.9	87.7	-45.7	-16.0

2018-2019 .
 (2)
 :
 (5,10 l/m²),
 20,80 l/m²
 l/m²,
 l/m²,
 48,20 l/m²,
 39,10 l/m²
 49,40 l/m²,
 23,20 l/m²,
 (43,80
 l/m²,
 18,90 l/m²) (Table 2).

During the agricultural year 2018-2019 there were significant deviations within the precipitation regime (Figure 2) in the months: December (rainfall in the amount of 15.1 l/m², deficit of 22.8 l/m²), February (with a rainfall value of 16.7 l/m² and a deficit of 12.9 l/m²), April (precipitation in the amount of 41.2 l/m², deficit of 17.8 l/m²), May (with a precipitation value of 134.4 l/m² and a deviation of 45.9 l/m²), August (precipitations in the amount of 174.6 l/m², deviation of 87.8 l/m²), September (precipitations in quantity of 17.0 l/m², deficit of 45.7 l/m²) (Table 2).



2. 2018-2019
Fig. 2. Rainfall evolution during 2018-2019 agricultural year

- 5 4 3
- :
- 1.1 *Medicago sativa* 100%
 - 1.2 *Medicago sativa* 75% + *Dactylis glomerata* 25%
 - 1.3 *Medicago sativa* 50% + *Dactylis glomerata* 50%
 - 1.4 *Medicago sativa* 25 % + *Dactylis glomerata* 75 %
 - 1.5 *Dactylis glomerata* 100%
 - 2.1 *Medicago sativa* 100%
 - 2.2 *Medicago sativa* 75 % + *Phalaris arundinacea* 25 %
 - 2.3 *Medicago sativa* 50% + *Phalaris arundinacea* 50%
 - 2.4 *Medicago sativa* 25 % + *Phalaris arundinacea* 75 %
 - 2.5 *Phalaris arundinacea* 100%
 - 3.1 *Medicago sativa* 100%
 - 3.2 *Medicago sativa* 75% + *Bromus inermis* 25%
 - 3.3 *Medicago sativa* 50% + *Bromus inermis* 50%
 - 3.4 *Medicago sativa* 25% + *Bromus inermis* 75%
 - 3.5 *Bromus inermis* 100%

2018 .

50 m 2000 m² (40 m). -

15 m², -

2 m. -

(Ene and Mocanu, 2016).

- :
- (3a);
 - 2 (3b);

The experiment is represented in 3 blocks with 5 variants and 4 replications. The following variants are included:

- 1.1 *Medicago sativa* 100 %
- 1.2 *Medicago sativa* 75 % + *Dactylis glomerata* 25 %
- 1.3 *Medicago sativa* 50 % + *Dactylis glomerata* 50 %
- 1.4 *Medicago sativa* 25 % + *Dactylis glomerata* 75 %
- 1.5 *Dactylis glomerata* 100 %
- 2.1 *Medicago sativa* 100 %
- 2.2 *Medicago sativa* 75 % + *Phalaris arundinacea* 25 %
- 2.3 *Medicago sativa* 50 % + *Phalaris arundinacea* 50 %
- 2.4 *Medicago sativa* 25 % + *Phalaris arundinacea* 75 %
- 2.5 *Phalaris arundinacea* 100 %
- 3.1 *Medicago sativa* 100 %
- 3.2 *Medicago sativa* 75 % + *Bromus inermis* 25 %
- 3.3 *Medicago sativa* 50 % + *Bromus inermis* 50 %
- 3.4 *Medicago sativa* 25 % + *Bromus inermis* 75 %
- 3.5 *Bromus inermis* 100 %

Establishment of the experimental field, was realized in the first decade of September 2018 and has a total area of 2000 m² (50 m wide and 40 m long). The surface occupied by a variant is 15 m² and the repetitions are delimited by access paths of 2 m wide.

The success of the sowing works depends on the care with the agrotechnical measures regarding the preparation of the germinating bed, the sowing time, the sowing depth, the distance and the sowing seed rate (Ene and Mocanu, 2016).

To establish the experiment, the following works were carried out:

- plowing the experimental surface (Figure 3a);
- preparing the seedbed with the rotary harrow by 2 perpendicular passes (Figure 3b);

-
 (3c);
 -
 (3d)
 (3).

: (, %),
 (%), (, %),
 (%), -
 (, %),
 (, %),
 (%),
 (%),
 (%),

(NIRS).

- delimitation and picketing the experimental field (Figure 3c);

- sowing the experimental variants (Figure 3d) with the established rates (Table 3).

Analysis of nutritional parameters: Crude Protein (CP, %), Ash (%), Crude Fiber (CF, %), Acid Detergent Fiber (ADF, %), Neutral Detergent Fiber (NDF, %), dry matter digestibility (DMD, %) and organic matter digestibility (OMD, %) was performed using the Near Infrared Spectroscopy (NIRS) technique.



a.



b.



c.



d.

. 3.

:
 ; b-

; c-

; d-

Fig. 3. Aspects from experimental field:

a-soil cultivation with lightweight disc harrow; b-seedbed preparing with rotary harrow; c-picketing of the experiment; d-manually sowing in rows.

3.

Table 3. Establishing mixtures of grasses and perennial legumes of grassland

Variant of the experiment	Sowing seed rate (kg/ha)	Variant of the experiment	Sowing seed rate (kg/ha)	Variant of the experiment	Sowing seed rate (kg/ha)
1.1	25	2.1	25	3.1	25
1.2	28	2.2	25	3.2	32
1.3	30	2.3	25	3.3	38
1.4	32	2.4	25	3.4	44
1.5	35	2.5	25	3.5	50

RESULTS AND DISCUSSION

The optimum period for harvesting perennial grasses is the ripening phase, and for perennial legumes is the flowering phase. Within this range a maximum quantity of nutrients is obtained, but which does not correspond to the moment when the maximum feed production is achieved.

After this phase, the following crops are harvested by mowing within a range of 5-6 weeks from the first mowing, depending on the weather conditions.

During the year 2019 there were 3 cycles of harvesting in the phenophase of earing the perennial grasses with the majority percentage of the mixture. Due to the climatic conditions, the interval between harvests was approximately 60 days.

Dry matter quantities on the mixtures under observation, are shown in Table 4, 5 and respectively 6 for cycle 1, 2 and respectively 3 cuttings of 2019. It is observed that, due to the adverse climatic conditions, the production from cycle 1 is relative lower than in cycle 2.

At first cut (Table 4), it can see that mixture 3.3 (*Medicago sativa* 50 % + *Bromus inermis* 50 %), 3.4 (*Medicago sativa* 25 % + *Bromus inermis* 75 %) and 3.5 (*Bromus inermis* 100 %) had the highest production, respectively 12.64, 12.93 and 12.81 t/ha Dry matter.

At second cut (Table 5), it can see that mixture 1.4 (*Medicago sativa* 25 % + *Dactylis glomerata* 75 %), 2.4 (*Medicago sativa* 25 % + *Phalaris arundinacea* 75 %)

(*Medicago sativa* 50 % + *Bromus inermis* 50 %), 3.4 (*Medicago sativa* 25 % + *Bromus inermis* 75 %) and 3.5 (*Bromus inermis* 100 %) 12.64, 12.93 12.81 t/ha

(*Medicago sativa* 25 % + *Dactylis glomerata* 75 %), 2.4 (*Medicago sativa* 25 % + *Phalaris arundinacea* 75 %)

% + *Phalaris arundinacea* 75 %) 2.5
 (*Phalaris arundinacea* 100 %) -
 - , 5.10,
 5.47 5.95 t/ha
 (6),
 1.1
 (*Medicago sativa* 100 %), 1.2 (*Medicago*
sativa 75 % + *Dactylis glomerata* 25 %)
 1.3 (*Medicago sativa* 50 % + *Dactylis*
glomerata 50 %) -
 , 1.16, 1.10 1.19 t/ha

and 2.5 (*Phalaris arundinacea* 100 %) had
 the highest production, respectively 5.10,
 5.47 and 5.95 t/ha Dry matter.

At third cut (Table 6), it can see that
 mixture 1.1 (*Medicago sativa* 100 %), 1.2
 (*Medicago sativa* 75 % + *Dactylis*
glomerata 25 %) and 1.3 (*Medicago*
sativa 50 % + *Dactylis glomerata* 50 %)

had the highest production, respectively
 1.16, 1.10 and 1.19 t/ha Dry matter.

4. (t/ha)

(2019 .)

Table 4. Dry matter (t/ha) at first cutting in 2019 for observed mixture

/Variant	1.1	1.2	1.3	1.4	1.5
RI	4.19	4.47	4.66	6.86	5.00
RII	3.44	3.73	2.29	8.54	4.44
RIII	5.53	6.75	1.72	5.24	4.44
RIV	4.53	4.23	4.69	5.81	5.98
/Average	4.42	4.80	3.34	6.61	4.96
/Variant	2.1	2.2	2.3	2.4	2.5
RI	4.36	5.00	5.64	5.63	5.96
RII	4.97	3.77	5.72	8.01	3.74
RIII	5.34	5.43	5.99	5.28	6.98
RIV	4.78	6.69	4.66	6.08	5.10
/Average	4.86	5.22	5.50	6.25	5.44
/Variant	3.1	3.2	3.3	3.4	3.5
RI	5.19	9.36	15.25	12.59	16.38
RII	3.65	14.08	9.78	10.41	12.18
RIII	4.70	12.13	11.96	11.58	10.89
RIV	5.70	9.16	13.58	17.16	11.78
/Average	4.81	11.18	12.64	12.93	12.81

5. (t/ha)

(2019 .)

Table 5. Dry matter (t/ha) at second cutting in 2019 for observed mixture

/Variant	1.1	1.2	1.3	1.4	1.5
RI	4.64	4.73	4.37	5.06	4.64
RII	4.03	3.72	4.05	5.16	4.37
RIII	4.49	5.18	5.47	5.72	4.59
RIV	4.54	5.26	5.30	4.45	5.20
/Average	4.42	4.72	4.80	5.10	4.70
/Variant	2.1	2.2	2.3	2.4	2.5
RI	3.90	4.47	4.64	5.87	5.94
RII	4.14	4.98	5.03	6.56	5.81
RIII	3.84	4.52	5.18	4.67	7.25
RIV	3.85	3.75	4.35	4.79	4.81
/Average	3.93	4.43	4.80	5.47	5.95
/Variant	3.1	3.2	3.3	3.4	3.5
RI	2.94	3.75	3.58	4.10	3.70
RII	4.79	4.25	2.32	4.09	2.39
RIII	4.59	3.24	3.82	4.68	3.69
RIV	4.68	2.63	2.70	4.28	3.72
/Average	4.25	3.47	3.10	4.28	3.37

6. (t/ha)
2019 .)

Table 6. Dry matter (t/ha) at third cutting in 2019 for observed mixture

/Variant	1.1	1.2	1.3	1.4	1.5
RI	1.22	1.30	1.27	0.97	0.36
RII	1.25	1.08	1.08	1.40	0.59
RIII	1.04	1.14	1.01	1.08	0.64
RIV	1.12	0.89	1.40	0.61	0.59
/Average	1.16	1.10	1.19	1.02	0.54
/Variant	2.1	2.2	2.3	2.4	2.5
RI	0.92	0.97	0.98	1.08	0.62
RII	0.90	0.91	0.88	1.12	0.81
RIII	1.11	1.33	1.28	1.12	1.09
RIV	0.91	0.66	0.87	0.73	0.49
/Average	0.96	0.97	1.00	1.01	0.75
/Variant	3.1	3.2	3.3	3.4	3.5
RI	1.06	0.90	0.50	0.63	0.37
RII	0.92	0.95	0.36	0.48	0.39
RIII	0.84	0.61	0.46	0.46	0.30
RIV	1.30	0.50	0.58	0.40	0.50
/Average	1.03	0.74	0.48	0.49	0.39

7

Dactylis glomerata
2019 .

Table 7 shows some elements regarding the quality of the forages from the experimental field, for all variants mixture with *Dactylis glomerata*, at first cutting in 2019.

7. *Dactylis glomerata* (%))

Table 7. Values of some elements regarding the quality of the forages, mixture with *Dactylis glomerata* (% DM)

/Variant	/CP	/Ash	/CF	/ADF	/ADL	/NDF	/DMD	/OMD
100%Ms	15.7	8.4	33.9	36.3	4.9	54.2	53.4	54.4
75%Ms+25%Dg	14.5	8.1	34.5	37.7	4.8	59.2	56.4	52.5
50%Ms+50%Dg	13.8	8.9	34.9	37.2	4.0	56.2	61.0	57.0
25%Ms+75%Dg	13.0	7.5	35.2	37.9	4.1	59.9	53.2	57.5
100%Dg	10.7	8.6	37.0	38.6	3.5	60.8	59.8	57.4

Ms-*Medicago sativa*; Dg-*Dactylis glomerata*

1.5 (*Dactylis glomerata* 100%),
(10.7%).
-
-
Medicago sativa -
100% (1.1).
1.2
(*Medicago sativa* 75% + *Dactylis glomerata* 25%),

For the variant 1.5 *Dactylis glomerata* 100%, the value of the CP was 10.7 %, this being the lowest value, the highest content having the variant 1.1 (*Medicago sativa* 100 %). It is also observed the influence induced by the forage legume in the mixture of variant 1.2 (*Medicago sativa* 75 % + *Dactylis glomerata* 25 %), the value of the crude protein content being high (14.5 %).

(14.5%).
 (25% 50%)
Medicago sativa
 13.0% (
 1.4) 13.8% (
 1.3).
 7,5%
 8.9%
 33.9% (*Medicago sativa* -
 100%) 37.0% (*Dactylis glomerata*
 100%).
 33.9% 35.2%.
 (36.3 %)
 1.1 (*Medicago sativa* 100%),
 (38.6%) 1.5 (100%
Dactylis glomerata).
 4,1% 4,9%.
 1.5 (*Dactylis*
glomerata 100%),
 3,5%.
 (54.2%) 1.1
 (*Medicago sativa* 100%).
Medicago
sativa 25 % + *Dactylis glomerata* 75 %
 (59.9%)
Dactylis glomerata 100% (60,8%).
 53. 2% (*Medicago*
sativa 25 % + *Dactylis glomerata* 75 %) 52,5%
 61.0% (*Medicago sativa* 50% +
Dactylis glomerata 50%)
 (*Medicago sativa* 75% + *Dactylis*
glomerata 25%) 57,5% (*Medicago*
sativa 25 % + *Dactylis glomerata* 75 %).
 8
Phalaris
arundinacea () 2019 .

Others two mixtures with the proportion of 25 % and 50 % *Medicago sativa* had relatively close contents 13.0 % for variant 1.4, respectively 13.8 % for variant 1.3.

The content in CA was between 7.5 % and 8.9 % in all the analyzed variants. In terms of CF content, the values ranged from 33.9 % to 37.0 %.

The highest value was within variant 1.5 (*Dactylis glomerata* 100 %), other variants registering values between 33.9 % and 35.2 %. The lowest value content in ADF was 36.3 % for variant 1.1 (*Medicago sativa* 100 %) and 38.6 % for variant 1.5 (100 % *Dactylis glomerata*).

The values of ADL content were relatively uniform, ranging from 4.1 % to 4.9 %, the exception being the variant 1.5 (*Dactylis glomerata* 100 %) with the lowest value of 3.5 %.

The content in NDF was 54.2 % and respectively 56.2 % for variant 1.1 and respectively variant 1.3.

The variants 1.4 and 1.5, had higher values. The DMD had values ranging from 53.4 % to 61.0 %. Values between 54.4% and 57.7% were recorded for the OMD.

Table 8 shows some elements regarding the quality of the forages from the experimental field, for all variants mixture with *Phalaris arundinacea*, at first cutting in 2019.

8.

Phalaris arundinacea (%)

Table 8. Values of some elements regarding the quality of the forages, mixture with *Phalaris arundinacea* (% DM)

/Variant	/CP	/Ash	/CF	/ADF	/ADL	/NDF	/DMD	/OMD
100%Ms	13.0	8.1	38.9	39.8	5.5	60.4	52.3	47.6
75%Ms+25%Pha	12.3	8.2	37.1	38.8	4.8	61.6	52.8	49.0
50%Ms+50%Pha	12.1	8.0	38.7	39.7	4.8	63.8	50.3	46.0
25%Ms+75%Pha	12.8	8.3	37.3	39.0	4.7	61.6	54.2	50.0
100%Pha	12.7	8.9	39.4	39.9	4.1	65.2	50.1	47.0

Ms-*Medicago sativa*; Pha- *Phalaris arundinacea*

(13,0%) 2.1
(*Medicago sativa* 100%).

12,1 12,8%.

8%. (39,9%)
2.5
(*Phalaris arundinacea* 100%),
(37,1%) 2.2 (*Medicago sativa* 75% + *Phalaris arundinacea* 25%).
Phalaris arundinacea 100%
(39,9%)

4,1 4,8%
2.1
(*Medicago sativa* 100%),
5,5%.
60,4% (*Medicago sativa* 100%)
65,2% (*Phalaris arundinacea* 100%).
9

Bromus inermis () 2019 .

The highest value of the crude protein content was 13.0 % registered at 2.1 variant (*Medicago sativa* 100 %). The other mixtures had very close CP values, ranging from 12.1 to 12.8 %. Homogeneous values also had the CA content, which is about 8 %. The highest value of the CF content was at variant 2.5 (*Phalaris arundinacea* 100 %) and the lowest value was 2.2 variant (*Medicago sativa* 75 % + *Phalaris arundinacea* 25 %). The variant 2.5 (*Phalaris arundinacea* 100 %) with the highest ADF content is noted. The ADL values are between 4.1 and 4.8 % for all the analyzed variants, except for the 2.1 variant (*Medicago sativa* 100 %) with a higher value (5.5 %). NDF has values between 60.4 % and 63.8 % in all variants, a higher value is observed in the variant 2.5 (*Phalaris arundinacea* 100 %).

Table 9 shows some elements regarding the quality of the forages from the experimental field, for all variants mixture with *Bromus inermis*, at first cutting in 2019.

9.

Bromus inermis (%)

Table 9. Values of some elements regarding the quality of the forages, mixture with *Bromus inermis* (% DM)

/Variant	/CP	/Ash	/CF	/ADF	/ADL	/NDF	/DMD	/OMD
100%Ms	13.6	8.2	36.3	38.7	3.2	59.0	60.5	49.8
75%Ms+25%Bri	9.7	8.6	38.9	43.0	4.5	69.5	55.5	45.0
50%Ms+50%Bri	9.0	7.2	39.0	43.0	4.2	69.0	53.6	44.7
25%Ms+75%Bri	9.4	8.0	39.4	42.7	4.3	69.1	54.2	45.7
100%Bi	8.0	6.7	39.3	42.4	4.2	68.8	50.1	47.0

Ms-*Medicago sativa*; Bri-*Bromus inermis*

8,0%
(*Bromus inermis* 100%) 13,6%
(*Medicago sativa* 100%).
3.4 (*Medicago sativa* 25% + *Bromus inermis* 75%) 3.2 (*Medicago sativa* 75% + *Bromus inermis* 25%),
(0,3%). 3.3 (*Medicago sativa* 50% + *Bromus inermis* 50%),
(9,0%)

5% 3.1

Regarding the content in CP, the values for the 5 variants of mixtures ranged from 8.0 % to 13.6 %. The highest value 13.6 % was recorded in the variant 3.1 (*Medicago sativa* 100 %), and the lowest value 8.0 % in the variant 3.5 (*Bromus inermis* 100 %). At variants 3.4 (*Medicago sativa* 25 % + *Bromus inermis* 75 %) and respectively 3.2 (*Medicago sativa* 75 % + *Bromus inermis* 25 %), the value of the protein content was close (9.4 respectively 9.7 %), while in the variant 3.3 (*Medicago sativa* 50 % + *Bromus*

(*Medicago sativa* 100%) 3.5
 (*Bromus inermis* 100%). 3.5
 (*Bromus inermis* 100%) -
 (6.7%). -
 3.2 (*Medicago sativa* 75% + *Bromus inermis* 25% - 8,0%)
 3.4(*Medicago sativa* 25% + *Bromus inermis* 75% - 8,6%). 3.3
 (*Medicago sativa* 50% + *Bromus inermis* 50%), 7,2%-
 7,2%.
 36,3% (*Medicago sativa* 100%) 39,4% (*Medicago sativa* 25% + *Bromus inermis* 75%).
 43,0% 42,4%
Medicago sativa *Bromus inermis*. -
 3.1
 (*Medicago sativa* 100%).
 3,2% 4,5%
 60,5% 3.1
 (*Medicago sativa* 100%), -
 50,1% 55,5%.
 68,8% 69,5%.
 3.1 (*Medicago sativa* 100%),
 -
 (59,0%).
 44,7% 49,8% -
Bromus inermis.

inermis 50 %), the value was slightly lower (9.0 %). A difference of about 5 % is observed between 3.1 variant (*Medicago sativa* 100 %) and 3.5 variant (*Bromus inermis* 100 %). In the case of mineral elements (CA), the lowest value (6.7 %), having a variant 3.5 (*Bromus inermis* 100 %), while the variants 3.2 and 3.4 having higher values, 8.0 % and respectively 8.6 %. The variant 3.3 (*Medicago sativa* 50 % + *Bromus inermis* 50 %), has a content of 7.2 %. The CF content had values between 36.3 % and 39.3 %. The lowest value 36.3 % was registered at variant 3.1 (*Medicago sativa* 100 %), the other variants having quite close values, between 38.9 % and 39.4 %. The ADF had values between 42.4 % and 43.0 % for all mixtures of *Medicago sativa* and *Bromus inermis*. The lowest value 38.7 % was recorded in the variant 3.1 (*Medicago sativa* 100 %). The values of ADL content were between 3.2 % and 4.5 % in all variants. The DMD was 60.5 % for the 3.1 variant (*Medicago sativa* 100 %), the other variants having values between 50.1 % and 55.5 %. The content of NDF, had for all mixture variants values between 68.8 % and 69.5 %, except for the variant 3.1 (*Medicago sativa* 100 %), which had the lowest content of 59.0 %. The values of OMD were between 44.7 % and 49.8 % for all 5 mix variants.

CONCLUSIONS

The mixtures used are simple, consisting of one grass and one forage legume, in which the participation percentage of the grasses increases from 25 % to 100 %, and the proportion of the perennial forage legume representing the difference.

Based on the chemical analysis, the mixtures of perennial grasses and legumes that are of superior quality are established and can be used in different stationary area conditions.

The aim of using these simple mixtures is that to obtain fodder with high

nutritional qualities and a high production, under conditions of climate change.

- The experiments have to be continued for a period of several years, in which the influence of climate change characterized by extreme phenomena will take place, following the stress resistance of the studied species.

/ REFERENCES

1. **Ene, T. A. and V. Mocanu**, 2016. Production, Conditioning and Storage of Perennial Grass and Legume Seeds of Grasslands - Technologies, Equipment and Installations. Capo-Lavoro Publishing House, Bra ov (Ro).
2. **Moga, I. and M. Schitea**, 2005 Modern Technologies for Seed Production in Fodder Plants. Ceres Publishing House, Bucharest (Ro).
3. **Schitea, M.**, 2002. Priorities for Scientific Research in Field Crops. Ceres Publishing House, Bucharest (Ro).
4. **Schitea, M.**, 2010. Results in the Improvement of the Alfalfa at INCDA Fundulea in the 2000-2009 period. *Annals of INCDA Fundulea*, LXXVIII (2), 63-78 (Ro).

full-sibs

„ , 7007 ,

Assessment of variability and phenotypic correlations between important agronomic and morphological traits in full-sibs alfalfa progenies

Diana Marinova

*Institute of Agriculture and Seed Science "Obraztsov chiflik", 7007, Rousse, Bulgaria
E-mail: diana27hm@abv.bg*

Original scientific paper

SUMMARY

2010-2013 .
" - .
9
(S/08-L, S/08- , S/08- , S/08- ,
S/08-R, S/08-S, S/08-B, S/08-V,
S/08-W)

The study was carried out at the experimental field of the Institute of Agriculture and Seed Science "Obraztsov chiflik", Rousse from 2014 to 2018. Nine polycross progenies (S/08-L, S/08- , S/08- , S/08- , S/08-R, S/08-S, S/08-B, S/08-V, S/08-W) of elite alfalfa plants whit native origin were included in the study. The progenies were created by free-limited pollination in polycross nursery. The aim of study was to evaluate phenotipic variability of main morphological and agronomical traits and the relationships among them at the selected alfalfa progenies as a source of the material for breeding programs.

- The field experiment in a randomized block design in four replications was performed. The alfalfa was grown without irrigation and the green mass in early flowering stage was harvested.
- The quantitative traits: dry matter yield, plants height and growth rate after cutting were

CV = 8,79%)
 (CV = 44,14% CV = 50,94%)
 (r = 0,788)
 (r = 0,824).
 (r = -0,288)
 (r = -0,546)
 (r = -0,685).
 (r = 0,924),
 e
 Fabaceae
 (Medicago sativa ssp.
 sativa),
 2n = 4x = 32.

(Radovi et al., 2009).

significantly influenced by the genetic factor environmental relationship and varied more widely during the study years than between progenies.

For the four-year period, the lowest phenotypic variability was determined for crude protein content (from CV = 1,39% to CV = 8,79%), and the highest one for dry matter yield (from CV = 44,14% to CV = 50,94%). A strong positive relationship between dry mass yield and plants height (r = 0,788) and regrowth rate (r = 0,824) was found.

The correlation coefficients shown a weak negative relationship of crude protein with dry matter yield (r = -0,288) and strong negative with plants height (r = -0,546) and regrowth rate after cutting (r = -0,685). It was found the plants height was strongly positively associated to regrowth rate (r = 0,924), as well as a tendency the correlation be stronger than this one of yield with the regrowth rate and plants height.

Key words: alfalfa, progenies, plants height, yield, variability, correlations

INTRODUCTION

Alfalfa is allogamous species of Medicago genus, Fabaceae family and it is the most widely grown perennial legume forage crop in the world. The common or blue alfalfa (*Medicago sativa* ssp. *sativa*), autotetraploid with 2n = 4x = 32 is primarily grown in our country.

The agronomic importance of alfalfa is based on its high potential for biomass production and the insurance of the cheapest source of protein rich forage of excellent amino acid composition and high digestibility animal husbandry (Radovi et al., 2009).

The alfalfa forage is rich with vitally important vitamins and microelements necessary for the normal growth and

(Marković et al., 2007).

development of animals (Marković et al., 2007). Alfalfa is the basic component in the feeding program for dairy and beef cattle, horses, sheep, birds and other animals.

- The perennial nature, high yield capacity, good adaptability to a wide range of ecological conditions and beneficial environmental impact increase the importance of alfalfa and favor its inclusion in the production of a number of valuable industrial materials (biofuel), its use for phyto-recovery of polluted soils and furthermore including in the menu of the people (Sanderson and Adler, 2008).

(Sanderson and Adler, 2008).

Over the years the alfalfa breeding programs are mainly focused on yield increasing, enhancing forage nutritive value and tolerance to abiotic/biotic stresses improving (Tucak et al., 2014).

(Tucak et al., 2014).

Worldwide the created alfalfa varieties differ in productive potential, forage quality, drought resistance, both in optimal and limiting conditions of the environment (Annicchiarico et al., 2010; Kertikova, 2014).

(Annicchiarico et al., 2010; Kertikova, 2014).

The agronomic and morphological traits variability in alfalfa are frequently used in breeding programs for developing varieties with a high forage productivity and better quality (Monirifar and Abdollahi, 2014).

(Monirifar and Abdollahi, 2014).

- There was reported high variability for many morphological and important agronomic traits in alfalfa (Mikić et al., 2005). Besides the traits variability among varieties there was established significant variability among individual plants within varieties/synthetic populations (Julier et al., 2000; Annicchiarico, 2006). The variability phenotypic expression of complex traits such as yield and forage quality is determined by both genetic effects and genotype environment interaction effects (Anderson et al., 1974;

(Mikić et al., 2005).

(Julier et al., 2000; Annicchiarico, 2006).

(Anderson et al., 1974; Babinec and Mikolaskova, 1992; Milic et al., 2010).
Churkova et al. (2016),

2020).

Tucak et al. (2008),

(Kertikova et al., 2003; Brummer, 2008; Basafa and Taherian, 2009).
Johnson et al. (1994),

Ibrahim et al. (2014),

o

Babinec and Mikolaskova, 1992; Milic et al., 2010). According to Churkova et al. (2016) in the breeding for forage quality of legumes it is important to study the variability under the genotypic factor influence in a specific region and regime of growing and use.

Regardless of growing conditions (drought stress or irrigation regime) plants height is one of the most promising traits for alfalfa genotypes selection with higher biomass quantity since by its phenotypic correlation it showed a high direct effect on the productive potential (Santos et al., 2020). A same result was reported by Tucak et al. (2008), who emphasized the meaning of plant height as an important criterion for the best genotypes choice at an early stage of selection.

In a number of publications was reported the rapid growth rate after harvest is an important criterion in the evaluation and selection of alfalfa genotypes (Kertikova et al., 2003; Brummer, 2008; Basafa and Taherian, 2009).

According to Johnson et al. (1994) the traits effecting forage yield and quality may interact directly or indirectly and should be studied for the purposes of breeding programs.

Ibrahim et al. (2014) also consider understanding the magnitude of the variability and the degree of the association between the different traits is important to provide a base for effective breeding.

The aim of study was to evaluate phenotypic variability of main morphological and agronomical traits and the relationships among them at selected alfalfa polycross progenies as a source of the material for breeding programs.

MATERIAL AND METHODS

2010-2013 .
 " " -
 26°02 43°48
 152 m.
 2,03% 2,17% (0 40
 cm).
 (- 5,84 5,94).
 (S/08-L, S/08-
 , S/08- , S/08- , S/08-R, S/08-S,
 S/08-B, S/08-V, S/08-W)
 ()
 ,
 ,
 -
 m²
 2,5 kg da⁻¹ 17.03.,
 12,5 m.
 ,
 13
 : 2010 . - , 2011 .
 2012 . - , 2013
 . -

The study was carried out from 2010 to 2013 in the experimental field at the Institute of Agriculture and Seed Science "Obraztsov chiflik" - Ruse. The institute is located at 43°48' N latitude and 26°02' E longitude in the northern climatic region of the Danube hilly plain.

The altitude is 152 m. The soil type is mainly leached and podzolic chernozem, located on sandy clay. The humus content is low and ranged from 2,03% to 2,17% (for the layer from 0 to 40 cm). The soil reaction is slightly acid (pH - from 5,84 to 5,94). The region has relatively low soil water permeability and satisfactory water preservation capacity, which is determined by the compacted transition horizon. The climate of the region has strong continental character.

Nine polycross progenies (S/08-L, S/08- , S/08- , S/08- , S/08-R, S/08-S, S/08-B, S/08-V, S/08-W) of elite alfalfa plants whit native origin were included in the study. The elites were selected on the basis phenotypic expression of main quantitative and qualitative characteristics – forage productivity, yield related traits, crude protein content, habit and leafiness. The progenies were created by free-limited pollination in polycross nursery.

The field **experiment** in a randomized block design in four replications and harvesting plot **size** of 10 m² was performed. Sowing was carried out on 17 of March with 2,5 kg da⁻¹ sowing rate and 12,5 cm interrow spacing. Alfalfa was grown without irrigation and the green mass in early flowering stage was harvested. For the study period a total 13 cuts were made, as follows: 2010 – two cuts, 2011 and 2012 – four cuts, and 2013 – three cuts.

The agronomical traits dry matter

yield, crude protein content, plants height and regrowth rate after cutting were estimated.

The yields (kg da⁻¹) by weighing the green biomass from each replicate at all regrowths were reported. To determine dry matter yield before each mowing, plant samples were taken, dried to constant weight and weighed.

The plants height (cm) was recorded by measuring the length of the stems from the soil surface to the top in 5 places in each harvesting plot.

Regrowth rate (cm) was determined, in centimeters, by measurement of plant height ten days after each harvest. Five measurements per each harvesting plot were made. The results were averaged to determine a mean plants height

Crude protein content in the dry mass was determined by the Kjeldahl method.

Variation and correlation analysis was performed to determine the magnitude of variation of the signs and the relationships between them. The STATGRAPHICS Plus software product was used to determine the coefficients of variation and correlation.

Kjeldahl.

STATGRAPHICS Plus.

RESULTS AND DISCUSSION

During the years of study, significant differences were observed in the temperature sums, amount of precipitation and their distribution during the regrowths development, which largely determined the variation of phenotypic expression of traits analyzed. Under meteorological conditions 2010 was relatively unfavorable for the alfalfa development. The total amount of precipitation during the autumn-winter period (October 2009 - March 2010) was close to the long-term norm for the region and allowed sowing to be carried out in

2010 .

a

2009 . -

(

2010 .)

2010 . -

2011 .

. K

-

1,

- the optimal time. The prolonged drought after sowing (April-May) suppressed growth and development of new establish alfalfa. The good soil moisture supplay in the period October 2010 - March 2011 ensured vigorous first regrowth during the second growing season.

- The amount of precipitation and temperature amounts for the period April-August were not differed significantly from those for the long term period and were contributed to the good development of alfalfa stands. The beginning of the third vegetation passed at good moisture supply and average daily temperatures favorable for normal development of the crop.

- Periods of prolonged rainfall with permanent drought alternated in the following months and the progenies failed to fully exhibit their potential on the traits analyzed. Meteorological conditions determined the last year as relatively unfavorable for alfalfa development.

- The results indicated that during the years of study the progenies ranked in different positions according to the traits analyzed. The coefficients of variation shown the degree of difference and magnitude of traits variation.

- Data on the traits variation between polycross progenies, presented in Table 1, shown a wide range of variability values.

1.

Table 1. Phenotypic variability of main agronomical traits between alfalfa progenies

/ Progenies	/ DMY, kg da ⁻¹	/ CP , %	/ PH, cm	/ RR, cm
2010				
S/08-L	446	20.56	49.3	13.78
S/08-A	472	19.21	56.4	15.60
S/08-M	393	21.10	49.7	13.92
S/08-X	497	19.13	54.1	16.00
S/08-R	564	19.50	53.9	16.50
S/08-S	463	19.63	52.8	15.22
S/08-B	511	19.56	54.5	15.80
S/08-V	467	19.94	49.5	14.78
S/08-W	500	20.31	50.4	15.75
/Mean	479.22	19.88	52.29	15.26
PCV	9.88	3.31	5.01	6.11
2011				
S/08-L	1937	19.94	72	16.73
S/08-A	1916	17.31	72	17.67
S/08-M	1933	17.44	75	17.15
S/08-X	2118	18.69	77	18.22
S/08-R	2010	17.75	73	18.00
S/08-S	1916	17.25	73	17.12
S/08-B	2078	17.25	73	19.10
S/08-V	1742	19.44	72	16.43
S/08-W	1977	20.56	72	17.95
/Mean	1958.56	18.40	73.22	17.60
PCV	5.54	7.03	2.34	4.70
2012				
S/08-L	1550	18.50	62	14.40
S/08-A	1413	17.38	60	13.70
S/08-M	1320	18.50	63	12.48
S/08-X	1507	17.56	64	13.90
S/08-R	1525	18.31	65	15.10
S/08-S	1405	17.88	63	14.55
S/08-B	1386	18.56	62	13.14
S/08-V	1346	18.60	58	12.66
S/08-W	1391	19.88	60	11.85
/Mean	1427.00	18.35	61.89	13.53
PCV	5.69	3.99	3.56	7.95
2013				
S/08-L	1658	18.56	71	15.90
S/08-A	1683	18.94	74	17.62
S/08-M	1750	20.48	76	18.54
S/08-X	1803	19.25	77	19.10
S/08-R	1945	18.63	79	17.86
S/08-S	1582	20.13	71	16.20
S/08-B	1709	18.31	73	17.50
S/08-V	1614	18.56	71	16.60
S/08-W	1676	20.25	72	15.90
/Mean	1713.33	19.23	73.78	17.25
CV	6.38	4.35	4.00	6.75

/ DMY – / Dry matter yield, / CP – / Crude protein content,
 / PH – / Plant height, / RR – / Regrowth rate

(CV = 9,88%).

(Kertikova et al., 2018).

a. (21,10%)
S/08-M, 20,56%
S/08-L 20,31%
S/08-W, 20,56%

3, 6, a (Kertikova et al., 2018).

52,29 cm half-sib
49,3 cm (S/08-L) 56,4 cm (S/08-).
(CV = 5,01%)

(CV=6,11%).

1958,56 kg da⁻¹
1742 kg da⁻¹ S/08-V
2118 kg da⁻¹ S/08-.

(CV = 5,54%),

S/08-W (20,56%), S/08-L (19,94%),
S/08-V (19,44%) S/08-X (18,69%)

- In the first year of study, a higher degree of variability under the genetic factor influence was observed regarding forage productivity. However, the reported values of the phenotypic variation coefficients for dry matter yield determined the variability between progenies as low (CV = 9,88%).

- Regarding crude protein content the highest values during the first vegetation was established, which corresponded to the biology of the crop (Kertikova et al., 2018). Deviations from this trend were observed in two progenies at the last year. With the highest protein content (21,10%) HS/08-M progeny distinguished followed by HS/08-L and HS/08-W with trait values 20,56% and 20,31%, respectively. The results obtained were close to those of Prista 3, Pleven 6, Dara and Dama varieties (Kertikova et al., 2018).

- The average plants height reported for the half-sib progenies studied was 52,29 cm at 49,3 cm minimum value (for HS/08-L) and 56,4 cm maximum value (for HS/ 08-A).

- The variation coefficient (CV = 5,01%) determined the degree of variability as very low. A low coefficient of phenotypic variation (CV = 6,11%) was also found for the regrowth rate after cutting.

- In the second year, the mean dry matter yield for the progenies was 1958,56 kg da⁻¹ at 1742 kg da⁻¹ minimum value in HS/08-V and 2118 kg da⁻¹ maximum one in HS/08-X.

- The differentiation of the half-sibs progenies was lower (CV = 5,54%) than in other years. Data shown that HS/08-W (20,56%), HS/08-L (19,94%), HS/08-V (19,44%) and HS/08-X (18,69%) were above average for progenies by the amount of protein content. The reported value for the variation coefficient shown that the trait varied the most strongly during the second growing season. The

CV = 4,7%

CV = 2,34%

1,

S/08-L (1550 kg da⁻¹),
S/08-R (1525 kg da⁻¹).
S/08- (1320 kg da⁻¹)

(CV = 5,69%)

11,85 cm
(S/08-W) 15,10 cm (S/08-R).

(CV = 7,95%)

(2012)

rtikova

half sibs

(CV = 4,00%)
(CV = 4,35%),
2,17

2

: results for plant height and regrowth rate CV = 2,34% and CV=4,7%, respectively were one-way with dry matter yield and shown less variability between progenies compared to the first year.

Data presented in Table 1, shown that during the third alfalfa growing season with the highest productivity HS/08-L (1550 kg da⁻¹) was distinguished, followed by HS/08-R (1525 kg da⁻¹). HS/08-M progeny had the lowest value (1320 kg da⁻¹) of the trait studied.

The magnitude of dry matter yield variability (CV = 5,69%) was similar to that in the second year. With respect to the stability of plants height and crude protein content no significant deviations were observed.

The reported values for regrowth rate shown a wide range of variability, from 11,85 cm (HS/08-W) to 15,1 cm (HS/08-R). The higher variability of the trait (CV = 7,95%) compared to other years was probably a result of the specific reaction of the progenies to the more uneven distribution of rainfall at the regrowths development during the growing season.

Kertikova (2012) found that rainfall has a more significant effect on the phenotypic expression degree of the trait than the variety, temperature and stand age.

The last year of study results confirmed the trend outlined for low variability of the analyzed characteristics between the half-sibs progenies studied.

Lower variability was observed for plant height (CV = 4,00%) and crude protein content (CV = 4,35%), with 2,17% magnitude of variation.

The variation coefficients mean for the study period, presented in Table 2, determined the variability at all traits between the progenies as weak.

2.

Table 2. Phenotypic variability of main agronomical traits for the study period

/Progenies	/ Dray matter yield, kg da ⁻¹				
	Mean	Min	Max	SD	CV
S/08-L	1397.75	446	1937	655.12	46.87
S/08-A	1371.00	472	1916	633.60	46.21
S/08-M	1349.00	393	1933	687.18	50.94
S/08-X	1481.25	497	2118	701.99	47.39
S/08-R	1511.00	564	2010	666.92	44.14
S/08-S	1341.50	463	1916	622.81	46.43
S/08-B	1421.00	511	2078	669.31	47.10
S/08-V	1292.25	467	1742	574.38	44.45
S/08-W	1386.00	500	1977	637.29	45.98
CV	4.93				
/Progenies	/ Crude protein content, %				
	Mean	Min	Max	SD	CV
S/08-L	19.39	18.50	20.56	1.03	5.29
S/08-A	18.21	17.31	19.21	1.01	5.52
S/08-M	19.38	17.44	21.10	1.70	8.79
S/08-X	18.66	17.56	19.25	0.77	4.13
S/08-R	18.55	17.75	19.50	0.73	3.95
S/08-S	18.72	17.25	20.13	1.38	7.35
S/08-B	18.42	17.25	19.56	0.95	5.15
S/08-V	19.14	18.56	19.94	0.67	3.52
S/08-W	20.25	19.88	20.56	0.28	1.39
CV	3.35				
/Progenies	/ Plants height, cm				
	Mean	Min	Max	SD	CV
S/08-L	63.58	49.3	72	10.53	16.56
S/08-A	65.60	56.4	74	8.71	13.28
S/08-M	65.93	49.7	76	12.32	18.69
S/08-X	68.03	54.1	77	11.12	16.35
S/08-R	67.73	53.9	79	10.86	16.03
S/08-S	64.95	52.8	73	9.18	14.13
S/08-B	65.63	54.5	73	9.05	13.79
S/08-V	62.63	49.5	72	10.83	17.29
S/08-W	63.60	50.4	72	10.46	16.45
CV	2,82				
/Progenies	/ Regrowth rate, cm				
	Mean	Min	Max	SD	CV
S/08-L	15.20	13.78	16.73	1.35	8.90
S/08-A	16.15	13.70	17.67	1.90	11.74
S/08-M	15.52	13.92	18.54	2.80	18.06
S/08-X	16.81	13.90	19.10	2.33	13.89
S/08-R	16.87	15.10	18.00	1.36	8.05
S/08-S	15.77	14.55	17.12	1.13	7.13
S/08-B	16.39	13.14	19.10	2.55	15.55
S/08-V	15.12	12.66	16.60	1.83	12.12
S/08-W	15.36	11.85	17.95	2.55	16.58
CV	3.97				

18,21%
 (S/08-) 20,25% (S/08-W).
 (CV = 2,82%),
 (CV = 3,97%).
 Tucak et al.
 (2014),
 22
 Avci et al. (2013).
 5 1
 7,27%; 5,5%
 6,48%. 16
 Harmanlioglu and Kaplan (2020),
 (1349,3 kg da⁻¹
 1878,86 kg da⁻¹),
 (55,75 cm 84,83 cm)
 (16,57%
 20,28%). Tucak et al. (2014),
 half-sibs
 (2).

Data shown the dry matter yield varied in a relatively broader range. The amount of protein content ranges from 18,21% (HS/08-A) to 20,25% (HC/08-W).

The variation analysis results shown that among the progenies the plants height varied less (CV = 2,82%) compared to the growth rate (CV = 3,97%).

The phenotypic variation coefficients found in the present study regarding average annual dry matter yield, plant height and regrowth rate after cutting were similar to data reported by Tucak et al. (2014) who examined the phenotypic performances of 22 selected alfalfa populations.

Our results were relatively lower than those obtained by Avci et al. (2013). In the study of 5 varieties and 1 alfalfa population, the authors reported coefficients of variation for dry matter yield, plant height and crude protein content 7,27%; 5,5% and 6,48%, respectively.

Studying 16 alfalfa varieties Harmanlioglu and Kaplan (2020) found a significant variation in dry matter yield (from 1349,3 kg da⁻¹ to 1878,86 kg da⁻¹), plant height (from 55,75 cm to 84,83 cm) and crude protein content (from 16,57% to 20,28%).

According to Tucak et al. (2014) besides the different genetic potential for many traits of alfalfa breeding materials, included in numerous investigations, the differences in data were probably in a great degree associated with the different environmental conditions where tests were conducted.

Phenotypic variation analysis data for the study period showed significant variability in the phenotypic expression degree of dry matter yield in all half-sibs progenies (Table 2).

It was found that the variation coefficients values at the progenies were significantly

CV = 50,94%),
 (CV = 44,14%
 S/08-R (1511 kg da⁻¹)
 (CV = 44,14%).
 S/08-W, (CV = 1,39)
 S/08- (CV = 8,79%)
 S/08-S, S/08-R (CV > 10%),
 S/08-L,
 S/08-
 (Tucak et al., 2008).

higher (from C = 44,14% to CV = 50,94%), compared to the this one reflecting the differences between them. This result of more pronounced seasonal differences confirmed the different responses of alfalfa progenies to changes in environmental factors for the conditions of the study. Data shown the highest value of the trait (1511 kg da⁻¹) established for HS/08-R was combined with lower variability (CV = 44,14%).

Regarding the amount of protein content in the dry matter, significant stability of the trait (CV = 1,39) for HS/08-W was found, during the study period. The progeny also distinguished by the highest crude protein content. The highest coefficient of phenotypic variation was obtained (CV = 8,79%) for HS/08-M. The established tendency for more significant variation of the yield over the years than between progenies was also observed for plants height. The coefficients values of phenotypic variation indicated the lowest trait variability in HS/08-A progeny, and the highest one at HS/08-M. The regrowth rate after cutting shown a medium magnitude of variability (CV >10%), in all polycross progenies, except HS/08-S, HS/08-R and HS/08-L. For these progenies were found slight trait variation. Data shown HS/08-M by the highest variability with respect to all traits analyzed was distinguished during period of study which is probably due to the stronger genetic factor environment interaction, led to more significant differences in the progeny response at regrowths and by years.

The knowledge about the relationships among traits is an important issue in plant breeding programs. The study of correlations provides the possibility of improvement of a larger number of traits simultaneously (Tucak et al., 2008). The best trade-off between forage quantity and quality is one of the most important targets in alfalfa breeding,

(Testa et al. 2011).
 (r = -0,514)
 (3).

since quantitative and qualitative traits are generally inversely correlated (Testa et al. 2011).

Phenotypic correlations among traits analyzed presented in Table 3 shown strong negative relationship between dry matter yield and crude protein content (r = 0,514) in first growing season of the progenies.

3.

Table 3. Coefficient of phenotypic correlation between main agronomical traits

2010					
	/DMY	C	/CPC	/PH	/RR
/DMY	1				
C /CPC	-0.517**		1		
/PH	0.485**		-0.848**	1	
/RR	0.897**		-0.801**	0.675**	1
2011					
	/DMY	C	/CPC	/P	/RR
/DMY	1				
C /CPC	-0.199		1		
/PH	0.576**		-0.249	1	
/RR	0.804**		-0.315	0.317	1
2012					
	/DMY	C	/CPC	/PH	/RR
/DMY	1				
C /CPC	-0.262		1		
/PH	0.512**		-0.312	1	
/RR	0.730**		-0.619**	0.641**	1
2013					
	/DMY	C	/CPC	/PH	/RR
/DMY	1				
C /CPC	-0.179		1		
/PH	0.952**		-0.013	1	
/RR	0.702**		-0.005	0.850**	1
2010 - 2013					
	/DMY	C	/CPC	/PH	/RR
/DMY	1				
C /CPC	-0.288		1		
/PH	0.788**		-0.546**	1	
/RR	0.824**		-0.685**	0.924**	1

** , * P 0.01 and P 0.05 / ** , * significant at P 0.01 and P 0.05, respectively
 /DMY – /Dray matter yield, /CP – /Crude protein content, /PH- /Plants height, /RR – /Regrowth rate

($r = -0,179$ $r = -0,262$)

, Testa et al. (2011)

and Abdollahi (2014) Monirifar
(2008), $r = -0,104$ $r = -0,11$ Tucak et al.
al. (2018) ($r = -0,751$). Cacac et

($r = -0,546$)
($r = -0,685$)

($r = -0,288$).

($r = 0,788$).

($r = 0,824$).

(Shanjani et al., 2013; Cacac et al., 2018).

e

Across next three years the negative correlation (from $r = -0,179$ to $r = -0,288$) was kept but low. The differences in the relationship power are probably due to the variable response of the progenies to fluctuations in temperatures and rainfall and their distribution in the formation of regrowth and in general over the years. Investigating the productive and qualitative characteristics of three alfalfa cultivars grown in the highlands of Southern Italy, Testa et al. (2011) found a strong negative relationship between protein content and temperature sums.

The correlation coefficients values between dry matter yield and crude protein content found in the present study were higher than those reported by Monirifar and Abdollahi (2014) and Tucak et al. (2008) $r = -0,104$ and $r = -0,11$; respectively and lower than this one ($r = -0,751$) determined by Cacac et al. (2018). The correlation coefficients between protein content with plants height ($r = -0,546$) and regrowth rate ($r = -0,685$), exhibited the same trend as that among crude protein and dry matter yield, but the negative relationship is more pronounced ($r = -0,288$).

Correlation analysis data shown a strong positive relationship between yield and plant height consistently over the years and mean for the study period ($r = 0,788$). It was found dry matter yield was positively related with regrowth rate ($r = 0,824$).

A similar trend regarding the relationship between yield and plant height and regrowth was reported in a number of studies (Shanjani et al., 2013; Cacac et al., 2018). It was established that the plants height was strongly and positively associated to regrowth rate, as well as a tendency the correlation be stronger than this one of yield with the regrowth rate and plants height.

and Taherian (2009).

Basafa

The phenotypic correlation coefficient determined was in line with this obtained by Basafa and Taherian (2009).

CONCLUSIONS

The quantitative traits dry matter yield, plant height and growth rate after cutting were significantly influenced by the genetic factor environmental relationship and varied more widely during the study years than between progenies.

For four-year period, the lowest phenotypic variability was found for crude protein content (from CV = 1,39% to CV = 8,79%), and the highest one for dry matter yield (from CV = 44,14% to CV = 50,94%).

A strong positive relationship between dry matter yield and plants height ($r = 0,788$) and regrowth rate ($r = 0,824$) was found.

The correlation coefficients showed a weak negative relationship of crude protein with dry matter yield ($r = -0,288$) and a strong negative with plants height ($r = -0,546$) and regrowth rate after cutting ($r = -0,685$).

It was found the plants height was strongly positively associated to regrowth rate ($r = 0,924$), as well as a tendency the correlation be stronger than this one of yield with the regrowth rate and plants height.

(CV = 1,39%
CV = 50,94%).

CV = 8,79%),
(CV=44,14%

(r = 0,788)
(r = 0,824).

(r = -0,288)

(r = -0,546)

(r = -0,685).

(r = 0,924),

/ REFERENCES

1. **Anderson, M. K., N. L. Taylor and R. R. Hill**, 1974. Combining Ability in *l*_o Single Crosses Red Clover. *Crop Science*, 14, 417-419.
2. **Annicchiarico, P.**, 2006. Diversity, Genetic Structure, Distinctness and Agronomical Value of Italian Lucerne (*Medicago sativa* L.) landraces. *Euphytica*, 148, 269-282.
3. **Annicchiarico, P., C. Scotti, M. Carelli and L. Pecetti**, 2010. Questions and Avenues for Alfalfa Improvement. *Czech Journal of Genetics and Plant Breeding*, 46, 1-13.
4. **Avci, M.A., A. Ozkose, A. Tamkoc**, 2013. Determination of Yield and Quality Characteristics of Alfalfa (*Medicago sativa* L.) Varieties Grown in Different Locations. *Journal of Animal and Veterinary Advances*, 12(4), 487-490.

5. **Babinec, J. and J. Mikolaskova**, 1992. Some Results of Breeding Proved in the Interstational Tests with Alfalfa. In: Proceedings of the X International Conference of the Eucarpia *Medicago* spp. Group, pp. 349-352.
6. **Basafa, M. and M. Taherian**, 2009. A Study of Agronomic and Morphological Variations in Certain Alfalfa (*Medicago sativa* L.) Ecotypes of the Cold Region of Iran. *Asian Journal of Plant Sciences*, 8, 293-300. DOI: [10.3923/ajps.2009.293.300](https://doi.org/10.3923/ajps.2009.293.300)
7. **Brummer, E.C.**, 2008. Alfalfa Improvement and the Maize Syndrome: Have We Suffered Enough. In: E. Piano (Ed.) Workshop for Pietro Rotili and the genetic improvement of alfalfa. CRA-FLC, Lodi. 55-69.
8. **Cacan, E., K. Kokten and M. Kaplan**, 2018. Determination of Yield and Quality Characteristics of Some Alfalfa (*Medicago sativa* L.) Cultivars in the East Anatolia Region of Turkey and Correlation Analysis between These Properties. *Applied Ecology and Environmental Research*, 16(2), 1185-1198. DOI: http://dx.doi.org/10.15666/aeer/1602_11851198
9. **Churkova, B., T. Bozhanska, and Y. Naydenova**, 2016. Feeding Value of Bird's-foot Trefoil (*Lotus corniculatus* L.) Cultivar under Conditions of the Central Northern Part of Bulgaria. *Banat's Journal of Biotechnology*, 7(14), 38-45.
10. **Ibrahim, H.I., N.M. Hamed and M.M. Abdel-Galil**, 2014. Genetic Behavior of Some Alfalfa Cultivars under New Valley Conditions. *Egyptian Journal of Plant Breeding*, 18(3), 495-507.
11. **Harmanlioglu, O. and M. Kaplan**, 2020. Herbage Yield and Quality Traits of Different Alfalfa (*Medicago sativa*) Cultivars. *Current Trends in Natural Sciences*, 9(17), 74-82. <https://doi.org/10.47068/ctns.2020.v9i17.008>
12. **Johnson, J.L.S., J.L. Hansen and D.R. Viands**, 1994. Relationships between Agronomic and Quality Traits in Alfalfa as Influenced by Breeding. In: Report of 34 North American Alfalfa Improvement Conference, 10-14 July, Ontario, Canada, pp. 17.
13. **Julier, B., C. Huyghe and C. Ecalte**, 2000. Within- and Among-Cultivar Genetic Variation in Alfalfa: Forage Quality, Morphology and Yield. *Crop Science*, 40, 365-369.
14. **kertikova, D.**, 2014. Evaluation of Lucerne Cultivars for Dry Matter Yield and Persistence. *Journal of Mountain Agriculture on the Balkans*, 17(1), 56-66.
15. **Kertikova, D.**, 2012. Study of Regrowth Rate of Alfalfa Depending on Factors Cultivar, Temperature and Rains. Scientific works of University of Ruse, 51, 51-55 (Bg).
16. **Kertikova, D., A.I. Ilieva, and T. Kertikov**, 2018. Crude Protein Content in Nine Alfalfa Cultivars and Its Relationship with Dry Matter Yield. *Journal of Mountain Agriculture on the Balkans*, 21(4), 83-92.
17. **Kertikova, D., C. Scotti, T. Kertikov and A. Atanassov**, 2003. Evaluation of Alfalfa Germplasm Resistant to Alfalfa Mosaic Virus (AMV). *Czech Journal of Genetic and Plant Breeding*, 39, 281-288.
18. **Markovic, J., J. Radovic, Z. Lugic and D. Sokolovic**, 2007. The Effect of Development Stage on Chemical Composition of Alfalfa Leaf and Stem. *Biotechnology in Animal Husbandry*, 23(5-6), 383-388.
19. **Mikic, V., J. Radovic, S. Mrfat-Vukelic, Z. Lugic, and D. Lazarevic**, 2005. Variability of Agronomic Characteristic in Eight Lucerne Genotypes. *Grassland Science in Europe*, 9, 565-568.
20. **Milic, D., S. Katic, J. Bocanski, D. Karagic, A. Mikic and S. Vasiljevic**, 2010. Importance of Progeny Testing in Alfalfa Breeding (*Medicago sativa* L.). *Genetika*, 42(3), 485-492. <http://www.doiserbia.nb.rs/img/doi/0534-0012/2010/0534-00121003485M.pdf>
21. **Monirifar, H. and N. Abdollahi**, 2014. Introducing Some Iranian Ecotypes of Alfalfa. *Journal of Plant Physiology and Breeding*, 4(1), 35-45.

22. **Radovi , J., D. Sokolovi and J. Markovi** , 2009. Alfalfa - Most Important Perennial Forage Legume in Animal. *Biotechnology in Animal Husbandry*, 25(5-6), 465-475.
23. **Sanderson, M. A and P. R. Adler**, 2008. Perennial Forages as Second Generation Bioenergy Crops. *International Journal of Molecular Science*, 9(5), 768-788.
24. **Santos, I.G., P. E. Teodoro; C. D. Cruz and R.P. Ferreira**, 2020. Impact of Water Deficit in the Relationship among Alfalfa Traits. *Bioscience Journal*, 36 (3), 905-913 <http://dx.doi.org/10.14393/BJ-v36n3a2020-41869>
25. **Shanjani, S.P., P. Salehi and A.A. Jafari**, 2013. Comparison of Phenotypic Trait Variation and Total Protein Polymorphism in Local and Exotic Germplasms of *Medicago sativa* in Iran, *New Zealand Journal of Agricultural Research*, 56(2), 142-155, DOI:10.1080/00288233.2013.781508
26. **Testa, G., F. Gresta and S.L. Cosentino**, 2011. Dry Matter and Qualitative Characteristics of Alfalfa as Affected by Harvest Times and Soil Water Content. *European Journal of Agronomy*, 34, 144-15.
27. **Tucak, M., S. Popovic, S. Grljusic, T. Cupic, V. Kozumplik and B. Simic**, 2008. Variability and Relationships of Important Alfalfa Germplasm Agronomic Traits. *Periodicum Biologorum*, 110(4), 311-315.
28. **Tucak, M., S. Popovic, T. Cupic, G. Krizmanic, V. Spanic, B. Simic and V. Meglic**, 2014. Agro-morphological and Forage Quality Traits of Selected Alfalfa Populations and Their Application in Breeding. *Turkish Journal of Field Crops*, 19(1), 79-83.

(*Plagionotus floralis* Pall.)

”

” -

”

”, 7007 ,

Annual Cycle of Development of Alfalfa Longhorn Beetle (*Plagionotus floralis* Pall.) at the Region of IASS “Obraztsov Chiflik” - Rousse

Evgeniya Zhekova

Institute of Agriculture and Seed Science “Obraztsov Chiflik”, 7007 Rousse, Bulgaria
-mail: e.d.zhekova@abv.bg

Original scientific paper

floralis Pall.

Plagionotus

SUMMARY

The alfalfa longhorn beetle *Plagionotus floralis* Pall. is a widespread alfalfa pest in Europe and the Middle East. In Bulgaria it is found in the whole country.

The observations were made during the period 2010-2015 at the experimental field of IASS “Obraztsov Chiflik” - Rousse.

A variation was determined at different levels of early onset and duration of development of the different phases of alfalfa longhorn beetle for 2010 - 2015, depending on the weather conditions during the years of the study.

The beginning of the pupa phase, for the period mentioned, was found in the first and second decade of May; the beginning of imago phase – from the third decade of May to the end of the second decade June; the laying eggs period – from the second ten days of June to the end of the first decade July; the hatching of larvae – from the third decade of June to the end of the second decade of July.

The average daily temperatures for the periods of occurrence of the different phases and for the previous ten-day periods are reliable criterion to predict their beginning of appearance.

The beginning of imago phase of alfalfa longhorn beetle, target of the chemical control, can be expected in the last ten days of May, first or second ten days of June, when the average daily temperatures for these and previous ten-day periods are around and above 19 °C.

Key words: alfalfa longhorn beetle, *Plagionotus floralis*, annual cycle of development, phenological calendar

Plagionotus floralis Pall.

(Katalin, 1960; Danilevsky, 2003; Sama et al., 2010; faunaeur.org/species_list.php, 14.12.2016).

Chorbadjiev (1930; 1932).

30-

floralis 1960-65

(1965) Makarov

The average daily temperatures for the periods of occurrence of the different phases and for the previous ten-day periods are reliable criterion to predict their beginning of appearance.

The beginning of imago phase of alfalfa longhorn beetle, target of the chemical control, can be expected in the last ten days of May, first or second ten days of June, when the average daily temperatures for these and previous ten-day periods are around and above 19 °C.

Key words: alfalfa longhorn beetle, *Plagionotus floralis*, annual cycle of development, phenological calendar

INTRODUCTION

Alfalfa longhorn beetle *Plagionotus floralis* Pall. is a widespread pest of alfalfa in Europe and the Middle East (Katalin, 1960; Danilevsky, 2003; Sama et al., 2010; faunaeur.org/species_list.php, 14.12.2016). The larvae are harmful, they feed on the inside of the central roots, as a result of which the damaged plants gradually dry out and the alfalfa fields become very thin.

In Bulgaria, alfalfa longhorn beetle was first reported in 1928 and 1929 by Chorbadjiev (1930; 1932). The author also points out data for a mass attack in the regions of Shumen, Pleven, Sofia, Plovdiv, Chirpan, Svilengrad and Panagyurishte.

After more than 30 years of absence in the entomological literature, *Pl. floralis* again became the subject of research in 1960-65 given the increased prevalence, number and damage by the pest in various settlements in our country. For the same period Makarov (1965) included alfalfa longhorn beetle in the lists of established harmful insects in alfalfa in the area of the Agricultural Research Institute "Obraztsov chiflik" near Ruse and published brief information about the morphology, biology, picture of damage,

(Makarov, 1968).

34
Pl. floralis.
 (Georgiev et al., 2002, 2003; Angelova et al., 2010),
 ”
 (Nikolova and Kertikova, 2008; Zhekova and Petkova, 2010; Petkova and Zhekova, 2012).

ways and means to combat it (Makarov, 1968).

- After this period, for 34 years, no
 - data on *Pl. floralis*. Information on the
 spread of the pest in new regions of the
 country (Georgiev et al., 2002, 2003;
 Angelova et al., 2010), as well as on the
 increase in the number and damages
 caused by alfalfa larvae in the area of
 IASS "Obraztsov chiflik", have been
 republished in recent years (Nikolova and
 Kertikova, 2008; Zhekova and Petkova,
 2010; Petkova and Zhekova, 2012).

These data suggest that alfalfa longhorn
 beetle has not lost its significance as a
 dangerous pest for alfalfa.

- This fact, as well as the scarce data in the
 - literature on the species, impose the need
 to continue research on the development,
 harmful activity and control of the pest in
 - the conditions of modern agricultural
 - practices and climatic conditions.

- The aim of this study is to trace the
 annual cycle of development (phenological
 development) of alfalfa longhorn beetle
 depending on climatic conditions during
 the years of study and to assess the
 possibility of average daily temperatures
 and the amount of rainfall to be used to
 predict the beginning of appearance of the
 individual stages of the development of
 - the pest.

MATERIAL AND METHODS

2010-2015 .

- The observations were made
 during the period 2010-2015 in the
 experimental field of the Institute of
 Agriculture and Seed Science "Obraztsov
 - chiflik" - Ruse. Alfalfa is grown under non-
 - irrigated conditions after a predecessor
 levelling crop oats. Established
 entomological methods have been used
 to determine the beginning and duration
 - of the individual stages of the life cycle of

alfalfa longhorn beetle.

To establish the end of the larva, respectively the beginning of the pupa stage, from the second half of April the roots of alfalfa plants are periodically removed, which are dissected for the presence of larvae or pupae of alfalfa longhorn beetle. After the discovery of the first pupa, route inspections revealed the beginning of the imagination of the beetles. The removal of plants and the dissection of roots continues until the larva and pupa are found in the roots, which is considered to be the end of the larva or pupa stage, respectively.

Numerical changes of adult insects were traced by the method of mowing with a standard entomological sweeping net (handle length 120 cm, hoop diameter 30 cm, bag depth 70 cm) (Mihaylova et al., 1982). In the alfalfa crops the survey was carried out diagonally, as in 2 places 25 swaths were made, and in the strips with weeds - in 2 places 5 swaths.

The samples were collected in the phenophase of alfalfa budding and the beginning of flowering in warm, quiet and sunny weather between 9 and 11 o'clock in the interval from 1-2 to 10 days depending on the climatic conditions. Supplementary feeding and copulation of adults were monitored in the field and in pot experiments in a vegetation house.

The beginning of egg laying has been observed since the beginning of June on test sites and on individual plants in the field, in pot experiments in a vegetation house and in a laboratory. Hatching of eggs was also observed in laboratory conditions.

To determine the beginning of hatching of larvae, periodically from the second half of June the roots of alfalfa plants were removed, which were dissected for the presence in them of larva of alfalfa longhorn beetle.

RESULTS AND DISCUSSION

Alfalfa longhorn beetle has one generation per year, which covers two calendar years and overwinters as a larva in the roots of alfalfa. The schemes for the beginning of occurrence and duration of development of the individual stages of the pest for the period 2010-2015 are presented in the phenological calendar (Figure 1) and clearly show their variation in the individual years depending on the climatic conditions.

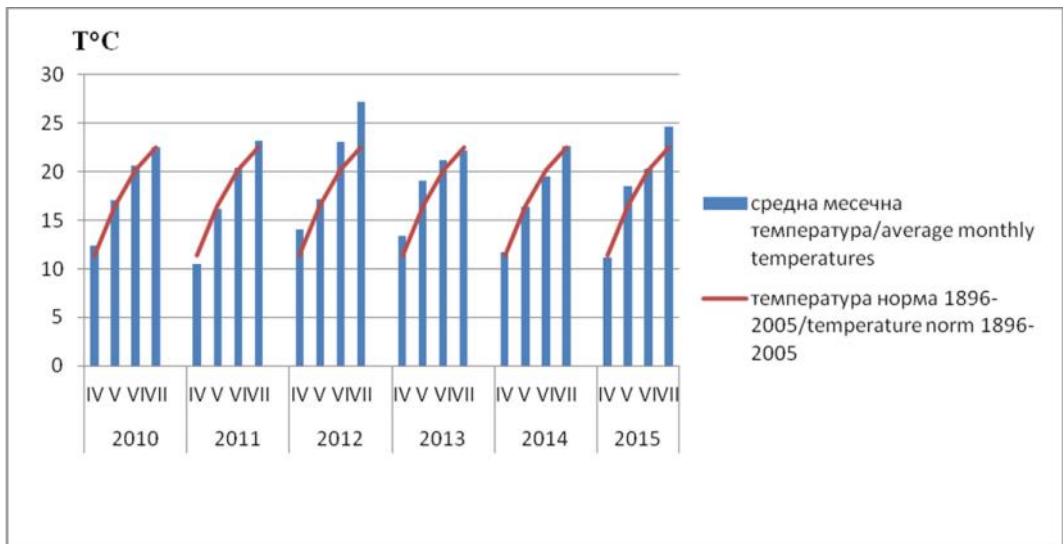
2010-2015 (1)

/Month	-IV	/May			/June			/July			VIII-XII
/Ten-day period		1	2	3	1	2	3	1	2	3	
/Year	2010										
/Phase	2010										
* /Larva											
/Pupa		0	0	0	0						
/Imago											
/Ovum								o	o		
** /Larva											
2011											
* /Larva											
/Pupa		0	0	0							
/Imago											
/Ovum								o	o	o	
** /Larva											
2012											
* /Larva											
/Pupa		0	0	0							
/Imago											
/Ovum								o	o		
** /Larva											
2013											
* /Larva											
/Pupa		0	0	0							
/Imago											
/Ovum								o	o	o	
** /Larva											
2014											
* /Larva											
/Pupa		0	0	0							
/Imago											
/Ovum								o	o	o	
** /Larva											
2015											
* /Larva											
/Pupa		0	0	0							
/Imago											
/Ovum								o	o	o	
** /Larva											
* -	/wintering generation; ** -					/new generation					

Fig. 1. Phenological calendar of alfalfa long-horned beetle, IASS "Obraztsov chiflik" - Ruse

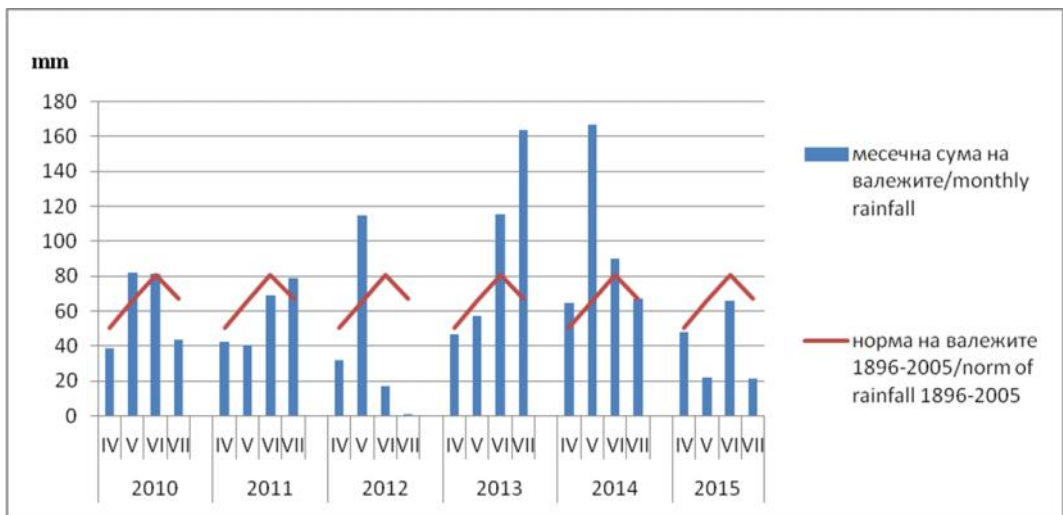
-
 2013 .
 -
 ,
 -
 (19,6° 19,1°
 15,2°C 13,2°)
 (0,6 1,0 mm) (2, 3).
 ,
 -
 -
 (14,4° -18,3°) -
 (7,0-63,0 mm)
 ,
 -
 2013 .
 (11,7° -14,1°),
 (11,4°),
 2011 . (2, 3).
 30-32 . 30-40 ,

- The earliest beginning of the pupa
 - stage during the years of the study was
 - noted in the first ten days of May 2013.
 Prerequisite for the earlier development of
 pupae are favourable climatic conditions
 for the period third ten days of April - first
 ten days of May, characterized by higher
 than the norm of average daily
 temperatures (19.6° and 19.1° at the
 norm of 15.2°C and 13.2° , respectively)
 and insignificant amount of rainfall (0.6
 and 1.0 mm) (Figure 2, 3).
 -
 In the remaining years studied, the
 beginning of the pupa stage of alfalfa
 longhorn beetle was registered in the
 second ten days of May. The analysis of the
 climatic conditions shows that the reason
 for the later development of the pupae is
 the lower average daily temperature
 (14.4° -18.3°) and the higher amount of
 rainfall (7.0-63.0 mm) for the first and
 second ten days of May, compared to the
 above values for the third decade of April
 and the first decade of May 2013.
 -
 A positive impact on the development of
 pupae of alfalfa longhorn beetle has the
 average monthly temperature for April
 (11.7° -14,1°), which for the years has
 exceeded the norm (11.4°), except for
 2011 (Figure 2, 3). The duration of the
 pupa stage for the study period was 30-40
 days, on average 30-32 days.



. 2. - 2010-2015, " (1896-2005)

Fig. 2. Average monthly temperatures and temperature norm (1896-2005) during the period April-July 2010-2015, IASS "Obraztsov chiflik" - Ruse



. 3. - 2010-2015, " (1896-2005)

Fig. 3. Monthly sums of precipitation and precipitation norm (1896-2005) during the period April-July 2010- 015, IASS "Obraztsov chiflik" - Ruse

.), (2011, 2012, 2014 (2010 .)

The beginning of imaging of adult insects was observed in the third decade of May (2013), the first (2011, 2012, 2014 and 2015) and the second (2010) decade of June. The data on the climatic conditions show that the average daily

18,8° 23,9° ,
 - 19,2° 21,1° . -
 (19,3° -22,7°),
 (9,3 38,4 mm
 10,1 109 mm)
 50-60
 18-26
 (2013 2015 .) (2012 .)
 (2010, 2011 2014 .),
 20,5° 24,1° (-
 21-22°)
 6,9 mm 55,7 mm.
 18,7° 24,2° ,
 19-20° 0,7-
 41,2 mm (2, 3).
 (2012, 2013 2015 .), (2010 .)
 (2011
 2014 .) 20-30
 (1).

temperatures for the indicated periods
 exceed the norm and vary from 18.8° to
 23.9° , most often from 19.2° to 21.1° .

Favourable for the uprise of the beetles
 are also the average daily temperatures
 for the ten-day periods preceding the
 imagination (19.3° -22.7°), which also
 differ in values above the norm. Due to
 the large difference in the amount of
 rainfall during the studied periods (from
 9.3 to 38.4 mm during the imagination of
 adults and from 10.1 to 109 mm for the
 previous ten-day period) no clear
 relationship was established with the
 beginning and duration of imagining.

The beetles fly for 50-60 days until the
 end of the second or end of the third
 decade of July. Immediately after imaging,
 they concentrate on the flowers of wild
 and cultivated plants, where they feed on
 the flower parts needed for their sexual
 maturation. Copulating pairs were observed
 from the second ten days of June to the
 second ten days of July for 18-26 days in
 different years. Copulation is multiple and
 is done by changing partners.

The egg-laying period for the
 studied six years was registered in the
 second (2013 and 2015) and third (2012)
 ten-day period in June, and the first ten-
 day period in July (2010, 2011 and 2014),
 characterized by an average daily
 temperature of 20.5° to 24.1° (most
 often 21-22°) and amount of rainfall from
 6.9 mm to 55.7 mm.

The climatic conditions during the
 previous ten-day period are close in
 values to the indicated average daily
 temperatures and vary from 18.7° to
 24.2° , most often 19-20° and the
 amount of precipitation is 0.7-41.2 mm
 (Figure 2, 3). The end of egg-laying was
 marked in the first (2012, 2013 and 2015),
 second (2010) and third ten-day period of
 July (2011 and 2014) with a duration of
 20-30 days (Figure 1).

- -
 - 310-320
 - (2010, 2011, 2012, 2014 .) 340
 - (2013, 2015 .)
 - (1).
 - (2013, 2015 .),
 - (2012 .)
 - -
 - (2010, 2011 2014 .)
 - 20,5-24° (21,2° -
 22,02°) 6,9
 55,7 mm. -
 -
 - 19° (2015 .) 26,8° (2012 .) (21,2° -22,02°)
 0,2 (2012 .) mm 39,8
 mm (2015 .) (2, 3).

The larva stage is the longest stage in the life cycle of alfalfa longhorn beetle - from about 310-320 days (2010, 2011, 2012, 2014) to 340 days (2013, 2015) depending on climatic conditions (Figure 1).

For the study period, their hatching was registered in the third ten days of June (2013, 2015), the first ten days of July (2012) and most often – in the second ten days of July (2010, 2011 and 2014) in average daily temperature for the previous ten-day period in the individual years of 20.5-24° (at norm 21.2° -22.02°C) and amount of rainfall from 6.9 to 55.7 mm. The temperatures for the ten-day periods during hatching are characterized by greater fluctuations – from 19° (2015) to 26.8° (2012) (at norm respectively 21.2° -22.02°C) and the amount of rainfall from 0.2 (2012) mm to 39.8 mm (2015) (Figure 2, 3).

2010-2015 .

CONCLUSIONS

- The beginning of appearance and duration of development of the individual stages of alfalfa longhorn beetle for the period 2010-2015 vary within different limits depending on the climatic conditions during the years of the study.

- The beginning of the pupa stage for the period is marked in the first and second ten days of May; the imagination of adult insects - from the third decade of May to the end of the second decade of June; of the egg-laying period - from the second ten-day period in June to the end of the first ten-day period in July; of larval hatching - from the third decade of June to the end of the second decade of July.

- The average daily temperatures for the indicated periods of occurrence of the individual stages and for the previous ten-day periods are a reliable criterion for predicting their beginning.

- The beginning of imagining of adult alfalfa longhorn beetles against which is directed chemical control, it can be

19°

expected during the last ten days of May, the first or second decade of June, when the average daily temperature for these and previous ten-day periods are around and above 19°C.

No clear relationship has been established between the sum of rainfall and the beginning of appearance of the individual stages of alfalfa longhorn beetle during the years of study and it cannot be used for forecasting purposes.

/ REFERENCES

1. **Angelova, V., Davidkov and D. Georgiev**, 2010. Changes in Species Composition of Beetles in Crop Pests (Insecta: Coleoptera) in Alfalfa. In: 20th Anniversary International Scientific Conference - Stara Zagora, Bulgaria, I, 217-221 (Bg).
2. **Chorbadzhiev, P.**, 1930. Notes on some Insect Pests of Cultivated Plants in Bulgaria in the Year 1928 and 1929. *Mitt. BULGAR. Ent. Ges.*, V, 63-106 (Bg).
3. **Chorbadzhiev, P.**, 1932. Information on Agriculture, XIII(1-2 and 3-4) (Bg).
4. **Danilevsky, M.**, 2003. Systematic List of Longicorn Beetles (Cerambycoidea, Coleoptera) of Europe. www.cerambyx.uochb.cz/list_europe.htm.
5. Fauna Europaea (www.faunaeur.org/species_list.php).
6. **Georgiev, G. and A. Stojanova**, 2003. New and Rare Longhorn Beetles (Coleoptera: Cerambycidae) in Strandzha Mountain, Bulgaria. *Acta Zoologica Bulgarica*, 55(2), 105-109.
7. **Georgiev, G., Stojanova, P. Bojadzhiev and Langurov**, 2002. Longhorn Beetles (Coleoptera: Cerambycidae) from Eastern Rhodopes in Bulgaria. *Forest Science*, 3/4, 115-119.
8. **Katalin, D.**, 1960. *Folia Entomologia Hungarica* (Series nova), XIII(18).
9. **Makarov, .**, 1965. Anniversary collection, AAS (Bg).
10. **Makarov, .**, 1968. Alfalfa Longhorn Beetle. *Plant Protection*, 2, 13-18 (Bg).
11. **Mihajlova, P., F. Straka and I. Apostolov**, 1982. Plant Protection Forecast and Signaling. Zemizdat, Sofia, 156-171(Bg).
12. **Nikolova, I. and D. Kertikova**, 2008. Comparative Evaluation of Lucerne Accessions According to Degree of Attack by Some Soil Insect Pests. *Journal of Mountain Agriculture on the Balkans*, 11(1), 48-59.
13. **Petkova, D. and E. Zhekova**, 2012. Forage and Seed Productivity in Alfalfa, Attacked by *Plagionotus floralis* Pall. (Coleoptera: Cerambycidae). *Bulgarian Journal of Agricultural Science*, 18(5), 708-712.
14. **Sama, G., J. Buse, E. Orbach, A. L. L. Friedman, O. Rittner and V. Chikatunov**, 2010. A new Catalogue of the Cerambycidae (Coleoptera) of Israel with Notes on Their Distribution and Host Plants. *Munis Entomology & Zoology*, 5(1), 1-55.
15. **Zhekova, E. and D. Petkova**, 2010. Productivity of Alfalfa Germ Plasm. New Data about Alfalfa Root Longhorn Beetle (*Plagionotus floralis* Pall.). *Banat's Journal of Biotechnology*, I(1), 56-60.