Bulgarian Journal of Agricultural Science, 29 (No 2) 2023, 351–358

Study on the chemical composition of alfalfa varieties attacked by *Uromyces striatus* Schroter

Iliana Ivanova[1*], Diana Marinova[1], Daniela Kertikova[2] and Anna Ilieva[2]

[1] *Agricultural Academy, Institute of Agriculture and Seed Science "Obraztsov Chiflik", 7000 Rousse, Bulgaria*
[2] *Institute of Forage Crops, 5800 Pleven, Bulgaria*
[*] *Corresponding author:* tri_dve@abv.bg

Abstract

Ivanova, I., Marinova.D., Kertikova, D. & Ilieva, A. (2023). Study on the chemical composition of alfalfa varieties attacked by *Uromyces striatus* Schroter. *Bulg. J. Agric. Sci., 29 (2)*, 351–358

During the period 2011-2018 a study on the changes in the biochemical composition of Bulgarian alfalfa varieties (Obnova 10, Pleven 6, Prista 2, Prista 3, Prista 4, Mnogolistna 1 and Victoria), infested by rust caused by the basidiomycete fungus *Uromyces striatus* (Schroter).

The study was conducted in the biochemistry laboratories of the Institute of Agriculture and Seed Science "Obraztsov Chiflik", Rousse and Institute of Forage Crops, Pleven.

It was found that: The obtained experimental results for the content of macroelements prove the negative influence of *Uromyces striatus* (Schroter) on the changes of some biochemical indicators in the leaves of the Bulgarian alfalfa varieties included in the experiment. Correlations were found between dependencies between the attack index of *Uromyces striatus* (Schroter) and the protein content ($r = 0.55$), sugars ($r = 0.36$), total phenols ($r = – 0.76$), saponins ($r = – 0.38$) and which prove that the disease affects the indicators that determine the quality of fodder. All Bulgarian alfalfa varieties included in the experiment can be conditionally defined as susceptible to rust (*Uromyces striatus* Schroter).

Keywords: Medicago sativa (alfalfa); resistance; rust; *Uromyces striatus* Schroter.

Introduction

Alfalfa (*Medicago sativa* L.) is one of the most important fodder legumes (Kertikova, 2008; Marinov-Serafimov et al., 2013; Hakl et al., 2014; 2016; Kulkarni et al., 2018; Vasileva & Kostov, 2018). Compared to other members of the legume family, it produces more protein per unit area with high nutritional value and is an excellent fodder for ruminants, it is included in the rations of poultry, rabbits and others (Kertikova, 2008; Baumont et al., 2014; Vasileva et al., 2017).

Fodder is a valuable source of minerals (Keskin et al., 2009; Vasileva & Pachev, 2015) and vitamins (Sottie, 2014), and the nitrogen content in plants varies depending on the varieties and the technology of cultivation and harvesting (Nikolova et al., 2018; Pypers et al., 2005; Luscher et al., 2014; Kusvuran et al., 2014; Kirilov et al., 2016).

The characteristics of the fodder depend on the structure of the plants, the chemical composition, the digestibility, the energy and the protein. The chemical composition of plants reflects the complex process of nutrition and growing conditions, characterizes the degree of nutritional security of plants, soil fertility and physiological availability of nutrients under specific growing conditions (Yancheva, 2002; Stamatov et al., 2015).

Fodder quality and crop longevity may be impaired by disease development (Gallenberg, 2002). One of them is the rust caused by the basidiomycete fungus *Uromyces striatus*

Sch. (Carlota Vaz Patto and Rubiales, 2014). Globally, the disease is widespread wherever alfalfa is grown.

According to many authors, it reduces yields and degrades fodder quality (Guan, 2000; Naseria & Marefatb, 2008; Samac et al., 2015; Qin et al., 2016). It attacks the leaves most often, but is sometimes found on stems and flower stalks. Rust-infested plants lag behind in growth (Kemen, 2004). Early leaf fall is observed, which leads to defoliation of plants, which affects the quantity and quality of fodder (Djukic, 2002). Li et al., 2018 reported poisoning of animals fed fodder from plants heavily infested with rust. There are changes in the content of nitrogen, phosphorus and potassium, proteins, phenols, water-soluble sugars, saponins and *in vitro* digestibility of the dry matter.

Nitrogen is contained in the composition of proteins (15-19% nitrogen), as well as in physiologically active substances that are involved in energy metabolism.

Phosphorus is involved in the construction of nucleoproteins. Improves inflorescence betting. Increases sugar content. Its deficiency causes reddening of the leaf stalks and accumulation of anthocyanins. Phosphorus decreases by 0.90% compared to healthy leaves.

Potassium affects the process of assimilation, the movement of carbohydrates in plants and the accumulation of sugars, increases resistance to pathogens and low temperatures. With potassium deficiency, slow growth is observed.

Saponins are terpene glycosides and cause swelling in animals, are often bitter in taste, reduce the taste of fodder and are toxic in certain concentrations (Abe et al., 1988). The content of saponins in plants is dynamic. The leaves of alfalfa are low in spring and autumn, and high in summer (Mugford et al. 2012; Osbourn et al. 2012; Abe et al., 1988; Foerster et al., 2006; Wina, 2005). Saponin synthesis in plants is induced in response to biotic stressors and pathogens, as a protective response of the plant (Kregiel, 2017).

Phenols are secondary metabolites. The ability to synthesize in the course of evolution allows plants to cope with the ever-changing challenges of the environment. They play a key role as protective compounds in stress factors such as low temperatures, pathogenic infections, nutrient deficiencies, play an important role in plant development, especially in lignin biosynthesis. (Bhattacharya et al., 2010). By synthesizing, releasing and accumulating phenols, plants respond to the attack of pathogens (Mert-Türk, 2002; Koornneef & Pieterse, 2008; Lu, 2009; Cushnie & Lamb, 2005; Taguri et al., 2006).

The aim of this study is to determine the effect of rust (*Uromyces striatus* Schroter) on changes in some biochemical parameters in Bulgarian alfalfa varieties.

Material and Methods

Accessions for analysis were collected from each alfalfa undergrowth. Phytopathological assessments were made during mass multiplication of the pathogen during the growth stage flowering of alfalfa plants by conventional methods. The reading is visual on a five-point scale, according to which with 0-healthy plants-without leaf spores, 1-resistant-single spores, 2-weakly sensitive-from 2-5 spores, 3-sensitive-spores occupy ½ from the surface of the leaf blade , 4- highly sensitive-spores occupy over ½ of the leaf blade.

Analyzes of plant accessions were performed by determining the chemical composition of the leaves, as they are the main organs of metabolism and changes in the supply of plants with nutrients affect their composition (Stamatov et al., 2015). Biochemical analysis of healthy and rust-infested plants was performed in the laboratories of Institute of Forage Crops, Pleven and Institute of Agriculture and Seed Science "Obraztsov Chiflik", Rousse. To determine the chemical composition of the aboveground mass, the plant samples taken were fixed for 15 minutes at 100^{o}C and dried to constant weight in a thermostat at 60^{0}C. Standard methods were used (AOAC, 2001). Phosphorus is determined – colorimetrically, by hydroquinone method; Potassium – on a flame photometer. The protein content was determined by Barnstein (Sandev, 1979), of total phenols by Swain & Hillis (1959), of water-soluble sugars by the method of Yermakov et al. (1987), of hemolytic saponins according to Jurzysta (1979) and *in vitro* digestibility of dry matter according to Aufrere & Graviou (1996). The attack index is calculated by Mc Kinney's equations. Depending on the% attack index, the studied varieties were grouped as follows: from 0 to 15 – highly resistant, over 15 to 35 – resistant, over 35 to 50 – moderately resistant, over 50 to 60 – sensitive and over 60 – highly sensitive. The yield of dry matter was reported and statistical data processing was performed by the method of analysis of variance.

The influence of rust on the productivity of the studied varieties was established by taking into account yield of dry matter per decare (kg/da). Statistical data processing was done through Anova for a one-factor experiment.

Results and Discussion

Analyzes of plant accessions are based on the determination of the chemical composition of the leaves, as they are the main organs of metabolism. The results show that the content of the macronutrients nitrogen, phosphorus and potassium changes under the influence of the pathogen. In those attacked by the pathogen, the content of macronutrients decreases, disproportionately to the degree of attack. The analysis of nitrogen content is determined by the fact that the macronutrient has the strongest influence on the ontogenetic development of alfalfa, and its deficiency causes disturbances in the dynamics of photosynthesis in plants. Nitrogen is contained in the composition of proteins (15 – 19% nitrogen), as well as in some physiologically active substances that are involved in energy metabolism. From the obtained results it is observed that the nitrogen decreases by 0.60%.

Phosphorus is involved in the construction of nucleoproteins. Improves inflorescence betting. Increases sugar content. Its deficiency causes reddening of the leaf stalks and accumulation of anthocyanins. Phosphorus decreases by 0.90% compared to healthy leaves.

Potassium affects the process of assimilation, the movement of carbohydrates in plants and the accumulation of sugars, increases resistance to pathogens and low temperatures. With potassium deficiency, slow growth is observed. Potassium decreased by 0.20% compared to healthy leaves (Table 1 and Table 2).

The data in Table 3 show that in rust-affected plants the protein content and *in vitro* digestibility of the dry matter are significantly reduced.

In healthy plants the protein content varies from 19.00% in cultivar Prista 4, Multileaf 1 and Obnova 10, to 22.00% in cultivar Victoria, and in sick plants from 15.96% in cultivar Multileaf 1 to 16.95% in cultivar Victoria. The protein content between samples from healthy and diseased plants decreased from 0.05 to 0.25%.

The *in vitro* digestibility of the dry matter decreases in diseased leaves from 6.0 to 10.0%.

The content of secondary metabolites (total phenols and saponins) and water-soluble sugars, which play a significant role in the protection of plants against pathogens, varies depending on the variety and is related to the resistance or susceptibility of a variety to this disease.

Under the influence of rust, the content of phenolic compounds in plants increases. The average data for the period show that the content of phenolic compounds is higher in diseased leaves than in healthy leaves.

This study found that it reduced the content of saponins in diseased plants. No link between phenol and saponin content was found in healthy plants. In diseased plants the correlation is negative. The content of saponins in alfalfa plants under normal conditions varies from 0.14 to 1.71%. With the progress of vegetation, aging of grass fodder and the development of the pathogen, digestibility decreases by 8.42%. The content of water-soluble sugars on average for the years

Table 1. Changes in the content of nitrogen, phosphorus, potassium depending on the attack by rust (*Ur. striatus*), average fot the period 2011-2018

Variants	Nitrogen content, %	Phosphorus content, %	Potassium content, %
First undergrowth			
Sturdy leaves	3.50	0.280	1.09
Sick leaves	–	–	–
r	0	0	0
Sturdy leaves	3.50	0.290	1.10
Sick leaves	–	–	–
r	0	0	0
Second undergrowth			
Sturdy leaves	3.30	0.240	1.09
Sick leaves	3.25	0.210	0.90
r	0.05	0.030	0.19
Sturdy leaves	3.08	0.206	0.95
Sick leaves	2.90	0.200	0.90
r	0.18	0.006	0.05
Third undergrowth			
Sturdy leaves	3.50	0.230	0.90
Sick leaves	3.25	0.215	1.09
r	0.25	0.015	0.19
Sturdy leaves	3.10	0.206	0.95
Sick leaves	2.90	0.200	0.90
r	0.20	0.006	0.05

Table 2. Nitrogen, phosphorus, potassium content of healthy and diseased leaves in percent of dry matter, average for the period 2011-2018

Variants	Nitrogen content,%	Phosphorus content,%	Potassium content,%
First undergrowth			
Sturdy leaves	4.80	0.30	1.40
Sick leaves^{+}	–	–	–
Second undergrowth			
Sturdy leaves	5.18	0.37	1.83
Sick leaves^{+}	–	–	–
Third undergrowth			
Sturdy leaves	4.80	0.32	1.45
Sick leaves	3.50	0.20	1.30
Fourth undergrowth			
Sturdy leaves	4.50	0.25	1.30
Sick leaves	3.20	0.18	1.10
Sick leaves^{+} there is no rust development in this undergrowth			

of the experiment increased in the samples from the diseased plants by 0.15% compared to the healthy ones.

Correlation relationships have been established between the attack index and the content of: proteins (r = 0.55), sugars (r = 0.36), total phenols (r = – 0.76) and saponins (r =

Table 3. Biochemical composition of alfalfa varieties attacked by *Ur. Striatus*, average for the period 2011-2018

№	Varieties/ Year	Protein from absolutely dry substance,%		Water soluble, %gars,%		Total phenols Relative units		Saponins from absolutely dry substance, %		Digestibility *in vitro* on dry matter of absolutely dry substance, %	
		Sick	Sturdy	Sick	Sturdy	Sick	Sturdy	Sick	Sturdy	Sick	Sturdy
	2011										
1	Obnova 10	16.02	19.90	1.65	1.70	0.195	0.180	1.49	1.50	65.80	72.30
2	Pleven 6	16.25+	21.00+	1.70+	1.60	0.198+	0.180	1.30	1.49	64.46	72.90+
3	Prista 2	16.72+	21.00+	1.70+	1.60	0.188	0.170	0.99	1.30	64.68	72.45+
4	Viktoriya	16.95+	21.00+	2.15+	1.90+	0.184	0.170	0.67	0.99	64.36	73.92+
5	Prista 3	16.35+	21.00+	1.65	1.50	0.212+	0.190	1.11	1.15	64.22	72.35+
6	Prista 4	16.57+	19.00	0.80	0.75	0.185	0.170	1.15	1.11	62.32	70.42
7	Mnogolist. 1	15.96	19.00	1.40	1.30	0.206+	0.190	1.38	1.15	61.11	71.07
	Average	16.40	20.27	1.57	1.47	0.190	0.170	1.15	1.24	63.85	72.20
	2012										
1	Obnova 10	16.02	19.90	1.65	1.60	0.185	0.180	1.49	1.50	65.80	72.50
2	Pleven 6	16.75+	21.00+	1.70+	1.65+	0.198+	0.160	1.30	1.49	64.46	72.10
3	Prista 2	16.72+	21.00+	1.72+	1.65+	0.178	0.170	0.90	1.30	64.68	72.45
4	Viktoriya	17.95+	21.00+	2.15+	1.90+	0.187+	0.177	0.60	0.99	64.36	73.92+
5	Prista 3	17.35+	21.00+	1.60	1.50+	0.210+	0.190+	1.11	1.15	64.22	72.35
6	Prista 4	16.59+	19.00	0.70	0.60	0.189+	0.178	1.15	1.19	62.32	70.52
7	Mnogolist. 1	15.98	19.00	1.20	1.00	0.200+	0.190+	1.38	1.56+	61.11	71.07
	Average	16.76	20.27	1.53	1.41	0.190	0.170	1.13	1.31	63.85	72.13
	2013										
1	Obnova 10	16.62	20.90	1.70	1.60	0.180	0.180	1.46	1.50	65.80	72.00
2	Pleven 6	16.25	21.00+	1.70	1.65+	0.190+	0.160	1.30	1.49	64.46	72.10+
3	Prista 2	16.70+	21.00+	1.70	1.65+	0.170	0.160	0.99	1.30	64.68	72.49+
4	Viktoriya	16.95+	22.00+	2.10+	1.90+	0.187+	0.177	0.67	0.99	64.36	73.92+
5	Prista 3	16.85+	21.00+	1.60	1.50	0.200+	0.190+	1.10	1.15	64.22	72.38+
6	Prista 4	16.57	19.00	0.90	0.60	0.180	0.173	1.10	1.19	62.32	70.52
7	Mnogolist. 1	16.50	19.00	1.20	1.00	0.200+	0.188+	1.30	1.56+	61.11	72.00
	Average	16.63	20.55	1.55	1.41	0.186	0.175	1.13	1.31	63.85	72.20
	2014										
1	Obnova 10	16.02	20.90	1.65	1.60	0.185	0.180	1.42	1.50	65.80	72.00
2	Pleven 6	16.25+	21.00+	1.70+	1.60	0.198+	0.180	1.30	1.49	64.46	72.10+
3	Prista 2	16.72+	21.00+	1.72+	1.65+	0.178	0.160	0.90	1.30	64.68	72.49+
4	Viktoriya	16.90+	22.00+	2.15+	1.90+	0.187+	0.177	0.67	0.99	64.36	73.92+
5	Prista 3	16.35+	21.00+	1.60	1.30	0.210+	0.190+	1.11	1.15	64.22	72.38+
6	Prista 4	16.90+	19.00	0.70	0.60	0.186	0.173	1.15	1.19	62.32	70.52
7	Mnogolist. 1	16.76+	19.00	1.20	1.00	0.200+	0.180	1.38	1.56	61.11	72.00
	Average	16.55	20.55	1.53	1.37	0.192	0.177	1.13	1.31	63.85	72.20
	2015										
1	Obnova 10	15.59	19.00	1.70	1.69	0.190	0.170	1.15	1.19	62.30	70.50
2	Pleven 6	16.15+	21.00+	1.70	1.60	0.190	0.180	1.30+	1.50+	61.10	71.00+
3	Prista 2	16.60+	21.00+	1.70	1.65	0.180	0.170	1.46+	1.50+	64.40+	72.40+
4	Viktoriya	16.70+	22.00+	2.00+	1.80+	0.187	0.167	0.90	1.00	64.36+	72.00+
5	Prista 3	16.25+	21.00+	1.60	1.50	0.200+	0.190+	1.10	1.15	64.00+	70.00
6	Prista 4	16.85+	19.00	0.90	0.60	0.190	0.170	1.10	1.19	62.32	70.50
7	Mnogolist. 1	16.76+	19.00	1.20	1.00	0.200+	0.180	1.38+	1.50+	61.00	72.00+
	Average	16.41	20.28	1.54	1.39	0.133	0.175	1.19	1.29	62.78	71.20

Table 3. Continued

	2016										
1	Obnova 10	16.00	21.00+	1.70	1.65	0.185	0.180	1.40	1.50	65.00	72.00
2	Pleven 6	16.25+	21.00+	1.70	1.60	0.190+	0.170	1.30	1.40	64.00	71.00
3	Prista 2	16.70+	21.00+	1.60	1.50	0.170+	0.160	1.00	1.20	64.60	72.00
4	Viktoriya	16.85+	22.00+	1.20	0.90	0.180	0.170	0.70	0.90	64.40	73,00+
5	Prista 3	16.30+	21.00+	1.60	1.50	0.200+	0.190+	1.15	1.30	64.20	72.30+
6	Prista 4	16.85+	19.00	0.90	0.60	0.187+	0.177	1.00	1.20	62.30	70.50
7	Mnogolist.1	16.70+	19.00	1.20	0.90	0.200+	0.190+	1.30	1.50	61.20	72.00
	Average	16.52	20.57	1.41	1.23	0.187	0.176	1.12	1.29	63.67	71.82
	2017										
1	Obnova 10	16.00	19.00	1.70	1.60	0.180	0.180	1.40	1.50	64.00	71.00
2	Pleven 6	16.20+	21.00+	1.70	1.60	0.190+	0.180	1.30	1.40	63.00	71.00
3	Prista 2	16.70+	21.00+	1.70	1.60	0.180	0.170	0.90	1.10	64.00	72.00+
4	Viktoriya	16.65+	22.00+	2.15+	1.90+	0.180	0.170	0.65	0.98	64.30+	73.00+
5	Prista 3	16.35+	21.00+	1.60	1.30	0.200+	0.180	1.11	1.00	62.30	70.50+
6	Prista 4	16.75+	19.50+	0.70	0.60	0.180	0.169	1.15	1.30	61.00	70.00
7	Mnogolist. 1	16.76+	19.00	1.53	1.37	0.180	0.170	1.13	1.30	63.40	72.00+
	Average	16.48	20.35	1.58	1.42	0.184	0.174	1.08	1.22	63.14	71.35
	2018										
1	Obnova 10	16.62	20.90	1.70	1.50	0.190	0.180	1.14	1.18	62.00	70.00
2	Pleven 6	16.10	21.00+	1.70	1.60+	0.193+	0.180	1.30+	1.50+	64.00+	71.00+
3	Prista 2	16.55+	21.00+	2.10+	1.90+	0.180	0.170	1.15	1.19	62.30+	70.00
4	Viktoriya	16.80+	21.00+	1.50	1.20	0.190	0.160	1.30+	1.50+	61.00	60.00
5	Prista 3	16.00	21.00+	0.80	0.60	0.192+	0.170	0.90	1.00	61.11	71.00+
6	Prista 4	16.80+	21.00+	0.70	0.60	0.180	0.160	1.10	1.15	64.60+	72.00+
7	Mnogolist. 1	16.50	19.00	1.53	1.37	0.200+	0.185	1.31+	1.50+	63.80+	72.00+
	Average	16.48	20.70	1.43	1.25	0.189	0.172	1.171	1.28	62.68	69.42
Average 2011-2018		14.46	20.44	1.51	1.36	0.181	0.173	1.137	1.28	63.45	63.97
Min.		15.59	19.00	0.70	0.60	0.170	0.160	0.60	0.98	61.00	60.00
Max.		17.95	22.00	2.15	1.90	0.212	0.190	1.49	1.56	65.80	73.92

Anova test: Protein. HSD[.05] = 24.15 HSD[.01] = 29.64, P = 0,003474; Water soluble sugars HSD[.05] = 27.05 HSD[.01] = 25.64 , P = 0,022257; Total phenols HSD[.05] = 24.05 HSD[.01] = 22.64; Saponins HSD[.05]=27.05 HSD[.01]=23.64, P = 0,022257

Table 4. One-way ANOVA

Source of variation	Sums of squares	Df	MS	F	F crit.	Effect of variation %
Index attack- Protein content						
Between groups	2696.05	1	2696.05	148.811	4.7472	92.5
Within groups	2913.46	12	18.117			7.5
Index attack – Sugar content						
Between groups	6345.27	1	6345.27	349.899	4.7472	96.7
Within groups	217.6144	12	18.1345			3.3
Index attack – total phenol content						
Between groups	6764.21	1	6764.21	374.729	4.7472	96.9
Within groups	216.61	12	18.051			3.1
Index attack- content of saponins						
Between groups	6473.22	1	6473.22	357.891	4.7472	96.7
Within groups	217.04	12	18.087			3.3
Index attack – *in vitro* dry matter digestibility						
Between groups	1357.33	1	1357.33	70.292	4.7472	85.4
Within groups	231.72	12	19.309			14.6

Table 5. Dry matter yield of the Bulgarian alfalfa varieties included in the experiment for the period 2011-2018

Varieties	Dry matter extraction																Total for 2011-2018	
	2011		2012		2013		2014		2015		2016		2017		2018			
	kg/da	%	kg/da	%	kg/da	%	kg/da	%	kg/da	%	kg/da	%	kg/da	%	kg/da	%	kg/da	%
Obnova 10	635.0	100.0	802.3	100.0	1421.5	100.0	850.0	100.0	1802	100.0	1400	100.0	906.0	96.1	800.0	100.0	7816	100
Pleven 6	654.8+	103.1	844.5+	105.3	1555.3^{+}	109.4	860.0+	101.1	1704	94.5	1712+	122.3	845.0	89.6	787.0	99.5	8961	114.6
Prista 2	712.0+	112.1	855.0+	106.6	1656.0^{++}	116.5	850.0	100.0	1639	90.9	1659+	118.5	863.0	91.5	771.0	95.9	9005	115.2
Viktoriya	590.3	93.0	808.0	100.7	1559.5^{+}	109.7	780.0	91.7	1796	99.6	1595+	113.9	896.0	95.0	804.0+	100.0	8828	112.9
Prista 3	718.8+	113.2	889.5+	110.9^{+}	1616.0^{+}	113.7	870.0+	102.3	1791	99.3	1490+	106.5	846.0	89.7	829.0+	103.1	8959	114.6
Prista 4	634.0	99.8	836.3+	104.2	1527.5+	107.5	780.0	91.7	1788	99.2	1480	105.7	853.0	90.5	864.0+	107.5	8762	112.1
Mnogolist. 1	663.5+	104.5	888.3+	110.7	1414.3	99.5	830.0+	97.6	1752	97.2	1339	102.8	845.0	89.6	801.0	100.0	8533	109.1
GD 5%	107.1		86.8		133.6		87.0		175.2		215 .0		106.3		94.9			
GD 1%	162.2		131.4		202.4		135.0		238.4		294.0		145.6		130.0			
GD 0.1%	260.7		211.3		325.3		215.0		100.9		401.0		198.4		177.2			

+доказана разлика; + proven difference;

Table 6. Rust attack index (in percent) *Ur. striatus* of the alfalfa varieties included in the experiment by years and on average for the study period 2011-2018

№	Varieties	Year								Average over the period, %	Category of sustain-ability
		2011	2012	2013	2014	2015	2016	2017	2018		
1	Obnova 10	38.75%	27.49%	44.50%	43.50%	39.00%	43.50%	44.00%	35.80%	45.22	S /sensitive
2	Pleven 6	27.49%	25.83%	45.00%	41.00%	30.50%	35.00%	41.00%	45.00%	41.54	S/sensitive
3	Prista 2	28.95%	36.25%	53.12%	53.00%	52.00%	50.00%	52.00%	50.00%	53.61	Highly sensitive
4	Viktoriya	31.87%	29.37%	48.95%	48.90%	45.00%	40.00%	40.00%	35.00%	45.54	S/sensitive
5	Prista 3	40.00%	28.12%	34.16%	34.86%	35.00%	30.00%	34.00%	40.00%	39.44	S/sensitive
6	Prista 4	45.83%	32.50%	42.08%	42.00%	45.00%	40.00%	42.00%	42.00%	47.34	S/sensitive
7	Mnogolist. 1	31.66%	23.33%	41.29%	41.00%	30.00%	40.00%	35.00%	41.00%	40.46	S/sensitive

– 0.38) and the ANOVA analysis performed (Table 4) show that the pathogen *Uromyces striatus* (Schroter) has an effect on the studied indicators determining the quality of fodder.

The influence of rust on the productivity of the studied alfalfa varieties was established, taking into account the amount of dry matter per decare (kg/da). The results presented in Table 5 show significant differences in dry matter yield over the years of study. On average for the period 2011-2018 the largest amount of formed dry biomass was reported for the variety Prista 3, and the lowest for the variety Victoria.

The analysis of Tables 5 and 6 shows that for the study period the varieties Prista 3 and Victoria included in the experiment formed the highest yield, regardless of the dynamics of meteorological factors, which determines tolerance to the disease in terms of varietal susceptibility.

It was found that for the three-year study period with the highest attack of the pathogen *Uromyces striatus* (Schroter) was reported in the variety Prista 2, while in the variety Prista 3 was relatively lowest.

Despite the established differences in the alfalfa accessions included in the experiment, a slight correlation was found with respect to the formed yield and the pathogen attack (in percentage) for the study period (r = -0.23).

The results obtained when reading the attack index show both varietal and age response of alfalfa. The average attack index for the period of the study varies from 39.44 for Prista 3 variety to 53.61% for Prista 2. The reported average annual results for the period 2011-2018. with regard to the attack by the pathogen characterize the varieties as highly sensitive to the *Ur. striatus* (Table 6).

Conclusions

The obtained experimental results for the content of macroelements prove the negative influence of *Uromyces striatus* (Schroter) on the changes of some biochemical indicators in the leaves of the Bulgarian alfalfa varieties included in the experiment. Correlations were found between dependencies between the attack index of *Uromyces striatus* (Schroter) and the protein content (r = 0.55), sugars (r = 0.36), total phenols (r = – 0.76), saponins (r = – 0.38) and which prove that the disease affects the indicators that determine the quality of fodder. All Bulgarian alfalfa varieties included in the experiment can be conditionally defined as susceptible to rust (*Uromyces striatus* Schroter).

References

Abe, I., Ebizuka, Y. & Sankawa, U. (1988). Purification of 2,3-oxidosqualene: cycloartenol cyclase from seedlings. *Chem. Pharm. Bull., 36,* 5031–4.

Aufrere, J. & Graviou, D. (1996). Prediction of the digestibility of forages and concentrated and compound feeds of herbivores by an enzymatic pepsin-cellulase method. Forages, 14. (Fr)

AOAC (2001). Official methods of analysis, 18-th ed. Association of Analytical Chemists. Gaithersburg, Maryland, USA.

Baumont, R., Heuze, V., Tran, G. & Boval, M. (2014). Alfalfa in ruminant diets. *Leg. Persp., 4,* 36-37.

Blazhev, V. (1982). Influence of diseases on productivity, quality and durability of alfalfa. *Agricultural Science, 3,* 91 – 95, (Bg).

Bhattacharya, A., Sood, P. & Citovsky, V. (2010). The roles of plant phenolics in defence and communication during Agrobacterium and Rhizobium infection. Oxford, UK: *Blackwell Publishing Ltd,* https://doi.org/10.1111/j.1364-3703.2010.00625.x.

Cushnie, T. & Lamb, A. (2005) Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents*, *26*, 342– 356.

Djukic, D. J. (2002). Plants for the production of fodder. Novi Sad, 224, (Sr).

Emil, J. C., Ghesquiére, M., Traineaau, R. & Mousset, C. (1997). Evaluation of the nutritional value of tall fescue genotypes obtained by different breeding strategies. *Fourrage, 151*, 373- 387, (Fr).

Yermakov, A., V. Arasimovich, N. Yarosh, I. Peruvian, G. Lukovnikov, M. Iconnikov. (1987), Methods for Biochemical Study of Plants. Moscow, Agropromizdat, 322-324, (Ru).

Foerster, H. *(2006).* MetaCyc Pathway: saponin biosynthesis I. *Retrieved, 23,* 1-10.

Frate, C. A. & Davis, R. (2007). Alfalfa diseases and management. Irrigated alfalfa management for Mediterranean and Desert zones. Chapter 10. wOakland: University of California Agriculture and Natural Resources Publication 8296.http://anrcatalog.ucdavis.edu.

Graham, P. (2008) Ecology of the root-nodule bacteria of legumes. *In:* Dilworth MJ, James EK, Sprent JI (Eds.), Nitrogen fixing leguminous symbiosis. *Springer Netherlands,* Europe, 23-58.

Gallenberg, D. (2002). Alfalfa disease management. *Plant Science,* SDSU, 1-3.

Guan, J. (2000). Assessing disease stress and modeling yield losses in alfalfa. Retrospective Theses and Dissertations. 12324. https://lib.dr.iastate.edu/rtd/12324.

Jurzysta, M. (1979). Haemolytic micromethod to rapid estimation of toxic alfalfa saponines. *Acta Agrobot., 32(1),* 5-11.

Julie, B. & Huighe, C. (1998). Genetic variability for Lucerne digestibility: relationship with dry matter production and leaf content. *Fourrage, 154,* 261-268, (Fr)

Hakl, J., Fuksa, P., Konečná, J., Pacek, L. & Tlustoš, P. (2014). Effect of applied cultivation technology and environmental conditions on lucerne farm yield in the Central Europe. *Plant Soil Environ., 60(10),* 475–80.

Hakl, J., Kunzová, E. & Konečná, J. (2016). Impact of long-term fertilization on crude protein fractions of lucerne forage in the first cut. *Grassland Science in Europe, 21,* 320–2.

Kemen, Er., Hahn, M., Mendgen, K. & Struck, Chr. (2004). Different Resistance Mechanisms of Medicago truncatula Ecotypes Against the Rust Fungus *Ur. Striatus*. *Phytopathology, 95(2-S.),* 153-157.

Keskin, N., Teksen, Y.A., Ongun, E. G., Ozay, Y. & Saygili, H. (2009). Does long-term talc exposure have a carcinogenic effect on the female genital system of rats? An experimental pilot study. *Arch. Gynecol. Obstet., 280(6),* 925-31. doi: 10.1007/s00404-009-1030-3.

Kertikova, D. (2008). The newest achievements in lucerne breeding in Bulgaria. *In*: *Proc. Breeding* 08, 24-27. Novi Sad, Serbia, 509–12.

Kirilov, A., Stoycheva, I. & Vasileva, V. (2016). Palatability of Annual and Perennial Legumes. *Nutrition and Food Science International Journal,* ISSN 2474-767X, 1(5). Available: https://www.researchgate.netpublication/311443558_Palatability_of_Annual_and_Perennial_Legumes 555573.

Kregiel, D., Berlowska, J., Witonska, Iz., Antolak, H., Proestos, Cha., Babic, M. & Babic, L. (2017). Saponin-Based, Biological-Active Surfactants from Plants, Application and Characterization of Surfactants, Reza Najjar, Intech Open, https://www.intechopen.com/chapters/54735.

Koornneef, A. & Pieterse, C. (2008) Cross talk in defense signaling. *Plant Physiol., 146,* 839– 844.

Kulkarni, K., Tayade, R., Asekova, S., Song, J., Shannon, J. & Lee, J. (2018). Harnessing the potential of forage legumes, Alfalfa, Soybean, and Cowpea for sustainable agriculture and global food security. *Front Plant Science*, *9,* 1314. doi:10.3389/fpls.2018.01314

Kusvuran, A., Ralice, Y. & Saglamtimur, T. (2014) Determining the biomass production capacities of certain forage grasses and legumes and their mixtures under Mediterranean Regional Conditions. *Acta Advances in Agricultural Sciences, 2,* 13-24.

Li, Y. Guangda, L., Haitao, Y., Xingzhi, J. & Kun, G. (2018). Antifungal Activities of Isoflavonoids from *Uromyces striatus* Infected Alfalfa. *Chemistry & Biodiversity, 15(12),* e180040. https://doi.org/10.1002/cbdv.201800407.

Lu, H. (2009) Dissection of salicylic acid-mediated defense signaling networks. *Plant Signal. Behav*., *4,* 713– 717.

Luscher, A., Harvey, M., Soussana, J. & Rees R. (2014) Potential of legume-based grassland-livestock systems in Europe: a review. *Grass Forage Sci., 69(2),* 206-228.

Marinov-Serafimov, Pl., Dimitrova, Ts. & Golubinova, I. (2013). Allelopathy – element of an overall strategy. *Acta Agriculturae Serbica, 18(35),* 23–37.

Mert-Türk, **F.** (2002) Phytoalexins: defense or just a response to stress? *Journal of Cellular and Molecular Biology Research, 1,* 1– 6.

Mugford, S. T. & Osbourn, A. (2012). Saponin synthesis and function. *In*: Isoprenoid Synthesis in Plants and Microorganisms; *Springer:* New York, NY, USA; 405–424.

Naseria, B. & Marefatb, A. (2008). Seasonal dynamics and prevalence of alfalfa fungal pathogens in Zanjan province, Iran. *International Journal of Plant Production, 2(4),* 327-340.

Nikolova, Iv., Georgieva, N. & Vasileva, V. (2018). Chemical composition in perennial fodder crops depending on the cultivation system and correlative dependencies with damage by root pests. *Journal of Mountain Agriculture on the Balkans, 21(4),* 93-105.

Pypers, P., Verstraete, S., Thi, C. & Merckx, R. (2005). Changes in mineral nitrogen phosphorus availability and salt-extractable aluminium following the application of green manure residues in two weathered soils of South Vietnam. *Soil Biology and Biochemistry, 37,* 163-172.

Samac, D. A., Rhodes, L. H. & Lamp, W. O. (2015). Compendium of Alfalfa Diseases and Pests, Third Edition. The American Phytopathological Society (APS). Websites verified 2/19/21.4023_S1.

Swain, T. & Hillis, W.E. (1959) The Phenolic Constituents of *Prunus domestica*. I.-The Quantitative Analysis of Phenolic Constituents. *Journal of the Science of Food and Agriculture, 10,* 63-68. http://dx.doi.org/10.1002/jsfa.2740100110.

Stamatov, I., Bozhinova, R. & Yancheva, D. (2015). Mineral composition of the leaves of new Oriental tobacco varieties, Krumovgrad 944 and Krumovgrad 17. *Soil Science, Agrochemistry and Ecology, 2,* 71-77, (Bg).

Sottie, E. T. (2014). Characterization of new sainfoin populations for mixed alfalfa pastures in Western Canada. A Thesis submitted to the school of graduate studies of the University of Lethbridge in Partial Fulfilment of the Requirements for the Degree Doctor of Philosophy, Lethbridge, Alberta, Canada.

Qin, F., Liu, D., Sun, B., Ruan, L., Ma, Z. & Wang, H. (2016). Identification of Alfalfa Leaf Diseases Using Image Recognition Technology. *PLoS ONE, 11(12*), e0168274. https://doi.org/10.1371/journal.pone.0168274

Taguri, T., Tanaka, T. & Kouno, I. (2006) Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure. *Biol. Pharm. Bull., 29,* 2226– 2235.

Vasileva, V. & Kostov, O. (2018). Performance of oats (*Avena sativa*) in response to preceding alfalfa (*M. sativa*) on mineral fertilization versus organic manuring on Chernozem soil. *Indian Journal of Agricultural Sciences, 88(3),* 416-419.

Vasileva, V. & Naydenova, Y. (2017). Nutritive value of forage biomass from mixtures of alfalfa with cocksfoot and tall fescue. *Journal of Global Innovations in Agricultural and Social Sciences, 5 (3)*, 121-129.

Vasileva, V. & Pachev, I. (2015). Root mass accumulation after different fertilization and soil cultivation of alfalfa (*M. sativa* L.). *Journal of Applied Sciences, 15(10),* 1245-1250.

Vance, C. P. (2001). Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. *Plant Physiol., 127(2),* 390-397.

Willis, W. G., Stuteville, D. L. & Sorensen, L. (1969). Effect of leaf and stem diseases on yield and quality of alfalfa forage. *Crop Sci., 9(5),* 637-640.

Wina, El., Muetzel, St. & Becker, Kl. (2005). The Impact of Saponins or Saponin-Containing Plant Materials on Ruminant. *J. Agric. Food Chem., 53(21),* 8093–8105.

Yancheva, D. (2002). Mineral composition of oriental tobacco leaves depending on nitrogen norm. Second Balkan Science Conf. "Quality and Efficiency of Tobacco Production, Processing and Processing". Plovdiv, 162-166, (Bg).

Received: **August, 10, 2021**; *Approved:* **December, 09, 2021**; *Published:* **April, 2023**