

## YIELD POTENTIAL OF ALFALFA CULTIVAR UNDER LITHUANIAN ENVIRONMENT CONDITIONS

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### Abstract

Seeking to achieve a balanced functioning of agrocenoses, it is vital to maintain a high productivity level of the crops grown with organic management practices, which major problem is yield losses due to poor soil quality. Alfalfa (*Medicago* spp.) is one of the most valuable forage crops of leguminous family, whose root system penetrates to deeper layers of the soil, improving physical and chemical soil properties. However, the use of alfalfa is limited due to its sensitivity to soil conditions. The aim of this study was to assess alfalfa's agro-biological properties under various soil conditions. Field experiments were conducted in four different locations with optimal management. Agro-biological properties of six alfalfa (*Medicago sativa* L. and *M. varia* Marth.) cultivars of various geographic origin were evaluated in a high productivity *Cambisol* and *Luvisol*. It was determined that cultivar 'Milda' distinguished for good overwintering, plants of cultivars 'Antanė', 'Malvina' and 'Skriveru' were taller at the beginning of flowering, cultivar 'Birutė' distinguished for the highest grass yield, while 'Antanė' and 'Birutė' distinguished for the dry matter (DM) yield. In a low productivity *Retisol*, the pH and mobile aluminium concentration had a major influence on overwintering, fresh matter and DM yields of the examined alfalfa cultivars. Alfalfa overwintering was 10.1–20.7% while fresh matter and dry matter yields were 2.0–2.4 and 2.8 times lower compared to alfalfa grown in high productivity soil.

**Keywords:** *Medicago* spp., agro-biological traits, productivity, soil type.

### Introduction

In Lithuania, agricultural land soils markedly differ in productivity. Nearly 40% of the soils are insufficiently fertile (Tripolskaja & Šidlauskas, 2010); moreover, those in the western part of the country are characterised by an acid reaction (Repšienė & Skuodienė, 2010). *Retisols* have a low buffering capacity and sorption, leading to rapid acidification, nutrient depletion, and reduced water retention (Repšienė & Karčauskienė, 2016). Acidic soils are the most significant barrier to agricultural productivity because of the direct effects of pH on root environment and plant growth (Tomchuk, 2018). Acidity limits root growth and, as a result, the absorption of water and mineral nutrients. The topsoil layer, which contains more organic matter, is dominated by  $H^+$ , while  $Al^{3+}$  toxicity is more evident in the layer below. Acidic soil conditions induce plant stress, leading to symptoms such as slowed growth, weak tillering, sparse crop coverage, delayed developmental stages, and heightened susceptibility to diseases (Goulding, 2016; Wang et al., 2016).

Alfalfa is valued for the crop longevity, high fertility, good forage quality and beneficial characteristics for the environment (improves soil physical properties, accumulates valuable nutrients and atmospheric nitrogen). Alfalfa distinguishes from the other legume grasses for its resistance to drought, because strong roots enable easier nutrients and water accessibility from deeper layers of the soil. Growing pure alfalfa crops or their mixtures together with the other grasses and legumes, the soil is enriched by nitrogen (Arlauskiene & Maikštėnienė, 2004). Therefore, alfalfa is a good forecrop (Skuodienė & Nekrošienė, 2012). Moreover, it is worth to plant alfalfa on the hill slopes of 10–20° steepness (Jankauskas & Jankauskienė, 2003).

Alfalfa, as well as the other legume grasses, prefers soil pH of 6.5–7.2. They can grow in more acid soil as well, where limy layer is not deep in the subsoil. However, in acid soils, alfalfa becomes more sensitive for overwintering, drought and diseases circulating during the vegetation period (Daugėlienė, 2010).

Not only in Lithuania, but also in other countries around the world, alfalfa breeding programmes focus on winter hardiness, seed and grass yields increase, crop longevity, and resistance to abiotic and biotic stresses. Various breeding and selection methods are currently being used to develop alfalfa varieties for different purposes, with higher grass and seed yields, resistance to diseases, tolerance to acid soils and mobile aluminium (Liatukienė et al., 2024).

When selecting alfalfa, it is very important to compare the new varieties being developed with those developed in Lithuania and in other countries. Foreign varieties of alfalfa have good grass and seed yields but are sensitive to the climatic conditions of our country (Liatukienė, 2012). In addition, the newly developed varieties must not only be higher yielding than previously developed varieties, but also distinguished by other desirable traits such as plant height at the beginning of flowering, leaf and inflorescence shape, colour, stem thickness, etc.

The study was aimed to evaluate and compare the productivity traits of alfalfa varieties in different genesis soils.

### Materials and Methods

#### *Experimental trial in the field*

The cultivars of alfalfa were sown in four locations of the experiment field. The first experiment field was at the Vėžaičiai Branch of Lithuanian Research Centre for Agriculture and Forestry (in the western part of

Lithuania). The soil of the experimental site was *Bathyglyeic Dystric Retisol* (WRB, 2022), with a texture of sandy light loam, high acid soil reaction,

phosphorus and potassium rich soil, organic carbon  $C_{org}$  1.23%, total nitrogen  $N_{total}$  0.12–0.14% and mobile Al 1.78–23.41 mg  $kg^{-1}$  (Table 1).

**Table 1**  
*Sites' characteristics*

<i>Indices</i>	<i>Experimental site</i>			
	<i>Vėžaičiai Branch</i>	<i>Institute of Agriculture</i>	<i>Utena PVTD</i>	<i>Pasvalys PVTD</i>
<i>Pedological indices</i>	<i>Retisol</i>	<i>Cambisol</i>	<i>Luvisol</i>	<i>Cambisol</i>
Soil type	<i>Retisol</i>	<i>Cambisol</i>	<i>Luvisol</i>	<i>Cambisol</i>
Soil texture	sandy light loam	light loam	sandy loam	clay loam
Sand, %	46.9	49.0	55.6	22.7
Silt, %	45.1	37.5	32.0	50.3
Clay, %	8.0	13.5	12.4	27.0
pH <sub>KCl</sub>	4.4–4.7	6.9–7.3	6.4	6.4
Mobile Al, mg $kg^{-1}$	1.78–23.41	0.00	0.00	0.00
Mobile P <sub>2</sub> O <sub>5</sub> , mg $kg^{-1}$	177–335	201–270	127	234
Mobile K <sub>2</sub> O, mg $kg^{-1}$	195–234	101–175	187	366
$N_{total}$ , %	0.12–0.14	0.14–0.16	0.13–0.15	0.15–0.16
Organic C, %	1.23	1.47	1.06	1.35
<i>Climatic indices</i>	2021			
Total annual precipitation, mm	907.7	515.7	623.0	795.2
Annual mean temperature, °C	7.4	7.4	6.9	7.9
Growing season's total precipitation, mm	528.1	388.5	433.6	526.6
Growing season's mean air temperature, °C	13.3	13.7	13.2	13.6
	2022			
Total annual precipitation, mm	803.2	628.4	807.0	642.9
Annual mean temperature, °C	8.1	8.0	7.3	7.7
Growing season's total precipitation, mm	461.4	472.1	579.8	427.1
Growing season's mean air temperature, °C	13.2	13.3	12.6	13.0
	2023			
Total annual precipitation, mm	1045.6	504.7	754.5	578.6
Annual mean temperature, °C	8.6	8.8	8.2	8.4
Growing season's total precipitation, mm	487.7	323.3	466.6	398.7
Growing season's mean air temperature, °C	13.8	14.4	13.8	14.1

Notes: Growing seasons from 4 to 10 months. PVTD – Plant Variety Testing Division.

The second experiment field was at the Institute of Agriculture of Lithuanian Research Centre for Agriculture and Forestry (Dotnuva, in the central part of Lithuania). The soil of the experimental site was *Endocalcari Epigleyic Cambisol* (WRB, 2022). The physicochemical properties of investigated soil were: light loam texture, neutral soil reaction, phosphorus rich soil and low amount of potassium in soil, organic carbon  $C_{org}$  1.47% and total nitrogen  $N_{total}$  0.14–0.16%. The soil of the experimental site in Utena (North-eastern part of Lithuania) was *Luvisol*, with a texture of sandy loam, neutral soil reaction, medium amount of phosphorus and potassium rich soil, organic carbon  $C_{org}$  1.06%, total nitrogen  $N_{total}$  0.13–0.15%. The soil of the experimental site in Pasvalys (North part of Lithuania) was *Cambisol*, with a texture of clay loam, neutral soil reaction, phosphorus and potassium rich soil, organic carbon  $C_{org}$  1.35%, total nitrogen  $N_{total}$  0.15–0.16%.

In order to evaluate agro-biological traits, alfalfa cultivars were sown on neutral (*Cambisol*) and low-yielding acid soil (*Retisol*). The seeds of six cultivars

were sown in 2020. During the period of 2021–2023, the agro-biological traits (overwintering, plant height before flowering, fresh and dry matter yields) were evaluated. The plants of alfalfa cultivars were cut three times in *Cambisol*; however, the plants were cut two times in *Retisol*, due to slow regrowth after cuts. In each location of the experiment, the seeds of alfalfa cultivar were sown in two rows of 3 m length in a randomized block design with three replications. Each alfalfa cultivar was sown in smaller experimental plots of 1.5 m<sup>2</sup> with a 1.0 m spacing between different genotypes. Weed control in alfalfa crops was managed using the herbicide Basagran 480 (active ingredient: bentazon 480 g L<sup>-1</sup>) at a rate of 2 L ha<sup>-1</sup>.

#### **Cultivar testing and registration**

In this experiment, Lithuanian alfalfa cultivars 'Antanė', 'Birutė', 'Malvina' and 'Žydrūnė', the cultivar 'Skriveru' from Latvia and a new Lithuanian cultivar 'Milda' (*Medicago varia* Martyn.) were used. In 2008, the cultivar 'Milda' has begun developed by hybridization method in the greenhouse conditions. Two cultivars ('Augūnė II' × 12991) were crossed between.

After the developing of the breeding population AI-3058 'Milda', this population was transferred to testing the value for cultivation and use (VCU) at the State Variety Testing Division in two locations of Lithuania – North-eastern (Utena State Plant Variety Testing Division) and North (Pasvalys State Plant Variety Testing Division). At the same time other testing of population AI 3058 'Milda' for DUS (Distinctness, Uniformity, Stability) was carried out in 2020–2023 at the Research Centre for Cultivar Testing (COBORU) in Poland. After the confirmation of positive DUS and VCU tests, in 2024, the cultivar 'Milda' was included to the Lithuanian National List of Plant Varieties as well as to EC Common catalogue of varieties of agricultural plant species.

#### **Weather conditions of experiment locations**

Lithuanian climate zone is semi-continental with moderately warm summers and moderately cold winters. The weather conditions of the experimental years in the central region of Lithuania (Dotnuva) were cold and wet in winter and very warm and humid in summer. Throughout the study period, the average air temperature was  $-1.1$  °C in January and  $19.5$  °C in July, with an annual precipitation of  $550$  mm, varying from  $505$  mm in 2023 to  $628.4$  mm in 2022 (Table 1). During the study period in the north-eastern region of Lithuania (Utena), the average air temperature was  $-1.7$  °C in January and  $18.9$  °C in July. Annual precipitation was  $728$  mm and ranged between  $623$  mm in 2021 and  $807$  mm in 2022 (Table 1).

In the north-eastern region of Lithuania (Pasvalys), the average temperature during the study period was  $-0.6$  °C in January and  $19.1$  °C in July. Annual precipitation was  $672$  mm and ranged between  $579$  mm in 2023 and  $795$  mm in 2021 (Table 1).

The western region of Lithuania (Vėžaičiai) distinguishes for the maritime climate and is characterized as moderately warm and humid. In comparison with the other regions, the amount of precipitation is the highest there. During the study period, the average air temperature was  $0.6$  °C in January and  $18.3$  °C in July. The average annual precipitation was  $908$  mm and ranged between  $803$  mm in 2022 and  $907$  mm in 2021 (Table 1).

#### **Statistical analysis**

The statistical analyses were performed using the statistical program SAS Enterprise Guide, version 7.13 (SAS Institute Inc., USA). The significance of the differences between experimental treatments was evaluated by one-factor analysis of variance (ANOVA). Tukey's HSD test was performed for multiple comparisons at the significant probability level  $p < 0.05$ .

#### **Results and Discussion**

Lithuanian National List of Plant Varieties contains 7 alfalfa (*Medicago sativa* L.) cultivars, 4 of them – Lithuanian. In 2024, a new sand alfalfa (*Medicago* × *varia* T. Martyn) cultivar 'Milda' was included. According to the studies of economic value in 2022–

2023, it was determined to be resistant to overwintering, crop flattening (Table 3). In the beginning of flowering, the plants were  $3.0$  cm taller, compared to the standard Latvian cultivar 'Skriveru'. 'Milda' was more yielding ( $1.93$  t ha<sup>-1</sup>) for the dry matter yield than 'Skriveru'. The indicators of forage quality were similar to the standard cultivar: proteins –  $20.0\%$  'Milda' and  $21.1\%$  'Skriveru', fibre –  $27.2\%$  and  $26.3\%$ , leafiness –  $45.8\%$  and  $45.2\%$ , respectively.

The results of the study showed that the overwintering, plant height before flowering, fresh matter yield and dry matter yield were significantly influenced ( $P < 0.0000$ ) by the soil pH<sub>KCl</sub>, and the weather conditions of experimental year (Table 2). According to the results of 2022–2023, cultivar 'Milda' overwintered very well (9 points). The overwintering of cultivars 'Antanė', 'Birutė', 'Žydrūnė', 'Malvina' and 'Skriveru' was similar to 'Milda' (ranged from  $8.6$  to  $8.9$  points). Our study showed that all cultivars of alfalfa differed by the plant height before flowering under different environmental conditions during the growing season in 2022–2023. Plant height is related to the environmental conditions and the genetics of individual genotypes (Kavut et al., 2014; Djaman et al., 2020).

In the vegetation period of 2022, contrastive weather conditions – rainy and warm dominated. The period of spring-summer in 2023 was less rainy, hotter and drier. Therefore, the cultivars of alfalfa were  $1.1$  times taller before flowering in 2022 than in 2023. During the growing stage in 2022 that was mentioned above, the height of plants of cultivar 'Milda' was similar to 'Antanė', 'Malvina' and 'Skriveru', and slightly lower compared to alfalfa cultivars 'Birutė' and 'Žydrūnė'  $1.05$  times, respectively. In 2023, alfalfa cultivar 'Milda' was taller than Latvian cultivar 'Skriveru', but lower than cultivar 'Žydrūnė'  $1.1$  times each, respectively.

Environmental conditions play a significant role in the variation of fresh and dry matter yields among alfalfa cultivars (Veronesi et al., 2010). Fresh matter yield of alfalfa cultivars was  $1.3$  times higher in 2022 than in 2023. The fresh matter yield of cultivar 'Milda' ( $47.6$  t ha<sup>-1</sup>) was similar to 'Birutė' ( $47.7$  t ha<sup>-1</sup>). 'Milda' was more yielding compared to cultivars 'Antanė' and 'Skriveru' by  $1.1$  times and was more yielding compared to 'Žydrūnė' and 'Malvina' by  $1.05$  times. In 2023, the fresh matter yield of cultivars 'Milda', 'Antanė', 'Žydrūnė' and 'Malvina' was similar and ranged from  $32.8$  t ha<sup>-1</sup> to  $37.0$  t ha<sup>-1</sup>, while the most yielding cultivar was 'Birutė' ( $40.6$  t ha<sup>-1</sup>).

The dry matter yield of alfalfa was similar in 2022 and 2023-  $8.8$  t ha<sup>-1</sup> and  $9.6$  t ha<sup>-1</sup>, respectively. Dry matter yield of cultivars 'Milda' and 'Žydrūnė' was similar in 2022, and these cultivars were more yielding compared to 'Skriveru', by  $1.1$  times. The dry matter yield of cultivars 'Antanė' and 'Birutė' was the highest and these cultivars were by  $1.4$  times more yielding compared to 'Skriveru' and by  $1.2$  times more yielding compared to 'Milda'.

**Table 2**

The ANOVA results for the soil  $pH_{KCl}$ , under field conditions effects on alfalfa wintering, height at before flowering, fresh and dry matter yields

Traits	Source		
	Cultivar	Soil $pH_{KCl}$	Year
Overwintering (score)	P<0.0000	P<0.0000	P<0.0000
Plant height before flowering (cm)	P<0.0000	P<0.0000	P<0.0000
Fresh matter yield (t ha <sup>-1</sup> )	P<0.0000	P<0.0000	P<0.0000
Dry matter yield (t ha <sup>-1</sup> )	P<0.0000	P<0.0000	P<0.0000

**Table 3**

The value for cultivation and use (VCU) of tested alfalfa cultivars in a high productivity soil in 2022-2023

Traits	2022 (2021 sowing year)		2023 (2021 sowing year)		2023 (2022 sowing year)	
	Pasvalys Plant Variety Testing Division					
	Milda	Skriveru	Milda	Skriveru	Milda	Skriveru
Overwintering (1–9 score)	9.0	9.0	9.0	9.0	9.0	9.0
Resistance to subsidence (1–9 score)	8.0	8.0	9.0	9.0	9.0	9.0
Plant height, cm	91.0	96.0	79.0	73.0	70.0	65.0
Leafiness, %	49.0	40.0	x	x	53.0	57.0
Crude protein, %	19.9	22.8	20.7	22.5	20.0	19.7
Crude fibre, %	30.6	28.7	26.4	26.0	26.3	26.6
Dry matter yield, t ha <sup>-1</sup>	22.0	17.7	21.0	17.6	19.5	16.8
Vegetation period (days until the first cut)	168.0	168.0	157.0	157.0	157.0	157.0
Utena Plant Variety Testing Division						
Overwintering, (1–9 score)	9.0	8.5	9.0	8.5	9.0	8.5
Resistance to subsidence (1–9 score)	7.3	7.6	9.0	9.0	8.5	8.5
Plant height, cm	72.0	68.0	63.0	59.0	64.0	60.0
Leafiness, %	33.3	32.1	x	x	47.9	51.5
Crude protein, %	18.7	20.7	18.9	20.7	21.8	20.0
Crude fibre, %	25.9	25.7	28.1	25.2	25.7	25.6
Dry matter yield, t ha <sup>-1</sup>	10.99	9.77	8.51	9.14	8.2	7.64
Vegetation period days until the first cut	178.0	178.0	167.0	166.0	166.0	166.0

Notes: x – not determined. 1 score – very low value, 9 score – very high value.

The alfalfa cultivars ‘Milda’, ‘Antanė’, ‘Žydrūnė’ and ‘Malvina’ were similar for their dry matter yield in 2023, respectively: 9.5 t ha<sup>-1</sup>, 9.7 t ha<sup>-1</sup>, 9.7 t ha<sup>-1</sup> and 9.8 t ha<sup>-1</sup>. The dry matter yield of 4 cultivars mentioned above was 1.3 times higher than ‘Skriveru’ but 1.2 times less yielding compared to ‘Birutė’.

The fresh matter and dry matter yields of alfalfa depended on growing conditions in productive soils of different regions of Lithuania. According to the economic value studies of 2022 and 2023 in Pasvalys (North part of Lithuania), the dry matter yield of cultivar ‘Milda’ was by 1.2 higher than the standard cultivar ‘Skriveru’. Studies in Utena (North-eastern part of Lithuania) showed, that dry matter yield of alfalfa ‘Milda’ was by 1.1 times higher than ‘Skriveru’ in 2022, while the yield of standard cultivar was similar in 2023.

In 2022, the dry matter yield of alfalfa ‘Milda’ was by a long way higher (2.7 times) in Pasvalys, than in

Dotnuva (central part of Lithuania). Cultivar ‘Skriveru’ was 2.4 times more yielding. In 2023, the dry matter yield of cultivar ‘Milda’ was 2.1 times higher in Pasvalys than in Dotnuva. In 2022, the dry matter yield of cultivars ‘Milda’ and ‘Skriveru’ was higher in Utena compared to Dotnuva, – by 1.3 times, respectively. Dry matter yield of cultivar ‘Milda’ in 2023 was higher in Dotnuva than in Utena – by 1.3 times.

Acidic soils are mostly related to high aluminium ions concentration there (Ožeraitienė, 2000; Repšienė & Karčauskienė, 2016). Aluminium ions interfere with various physiological and cellular processes in the roots. High concentrations of mobile Al are toxic to plants and cause oxidative stress and may disrupt metabolic functions of plants (Langer et al., 2009; Bartoli et al., 2017). However, in acidic soil with small concentrations of mobile Al can sometimes increase plant growth or bring other desirable effects (Kisnerienė & Lepeikaite, 2015). Root function is

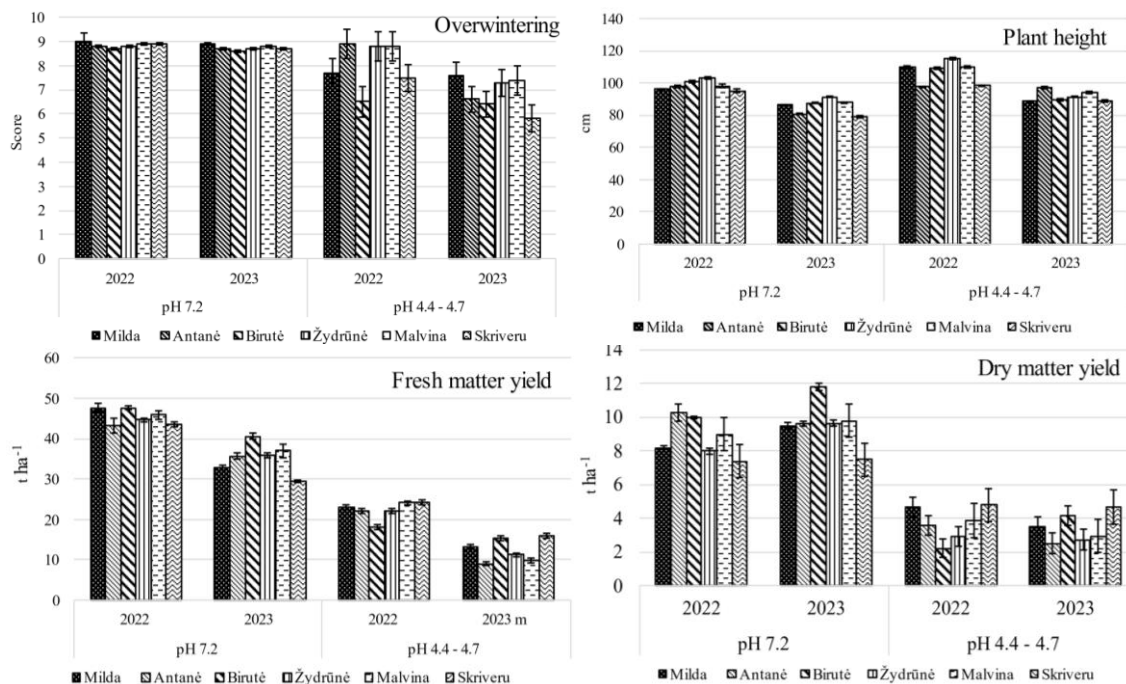
extremely dynamic and dependent on internal plant signals, root traits and morphology, and the physical, chemical and soil environment (Kuka et al., 2016). Our study showed that overwintering of alfalfa cultivars in acidic soil with mobile Al ( $13.6 \text{ mg kg}^{-1}$ ) concentration, was better (by 1.2 times) in the second winter than in the third winter. Overwintering of cultivars ‘Milda’ and ‘Skriveru’ was by 1.2 times worse in the second winter compared to cultivars ‘Antanė’, ‘Žydrūnė’ and ‘Malvina’. In the third winter, cultivars ‘Milda’, ‘Žydrūnė’ and ‘Malvina’ persisted

(overwintered) similarly, and they overwintered by 1.3 times better than ‘Skriveru’.

The height before flowering of studied alfalfa cultivars was by 1.2 times higher in the second year of use than in the third year of use. The cultivars ‘Milda’, ‘Birutė’ and ‘Malvina’ were taller by the height before flowering in the second year of use than ‘Antanė’ and ‘Skriveru’, by 1.1 times. Alfalfa ‘Birutė’ distinguished for the plant height (115.3 cm). In the third year of use, ‘Milda’, ‘Birutė’ and ‘Skriveru’ were by 1.1 times taller at before flowering compared to ‘Antanė’ and ‘Malvina’.

**Figure 1**

*Comparison of agro-biological traits of alfalfa in productive and less productive soil*



Notes: pH – soil acidity ( $\text{pH}_{\text{KCl}}$ ) by the potentiometric method in the extraction of 1 M KCl. Vertical dashes indicate the mean of standard error.

The fresh matter yield of the alfalfa cultivars was 1.8 times higher in the second year of use than in the third year of use, because grasslands of alfalfa thinned. The fresh matter yield of the second year of use of cultivars ‘Malvina’ and ‘Skriveru’ was similar to ‘Milda’, while it was by 1.1 times higher than ‘Antanė’ and ‘Žydrūnė’ and by 1.3 times higher than ‘Birutė’.

In the third year of use, the fresh matter yield of alfalfa ‘Birutė’ and ‘Skriveru’ was higher compared to cultivars ‘Milda’ – by 1.2, ‘Žydrūnė’ – by 1.4 and ‘Malvina’ – by 1.6 times. The highest dry matter yield of the alfalfa cultivars ‘Milda’ and ‘Skriveru’ ( $4.7 \text{ t ha}^{-1}$  and  $4.8 \text{ t ha}^{-1}$  respectively) was in the second year of use. It was by 1.2 times higher than cultivars ‘Antanė’ and ‘Malvina’. In the third year of use, the alfalfa cultivar ‘Skriveru’ distinguished for the highest dry matter yield ( $4.7 \text{ t ha}^{-1}$ ). This cultivar was more yielding than ‘Milda’ – by 1.3 times, ‘Žydrūnė’ – by 1.7 times and ‘Antanė’ – by 1.9 times.

In summary of the average data, in less productive soils the amount of overwintered alfalfa plants in the second winter was lower by 10.1%, and in the third winter it was lower by 20.7% than in productive soils ‘Figure 1’. Analysed alfalfa plants of the second year of use in the beginning of flowering were by 8.2% taller in less productive soils, and in the third year of use – taller by 7.1% compared to alfalfa that was growing in productive soils ‘Figure 1’. The fresh matter and dry matter yields of examined alfalfa of the second year of use in less productive soils were 2.0 and 2.4 times lower, and in the third year of use – 2.8 and 2.8 times lower than in productive soils ‘Figure 1’. Alfalfa spread is limited by the soil acidity and the abundance of moisture in the soil (Daugėlienė, 2010).

### Conclusions

1. New alfalfa cultivar ‘Milda’, similarly as the other examined cultivars distinguished for the high and

stable fresh matter and dry matter yields and are adapted to the conditions of Lithuanian climate.

2. The fresh matter and dry matter yield of the examined alfalfa cultivars on average in three years were 40.4 and 9.2 t ha<sup>-1</sup> in productive soils and 17.4 and 3.6 t ha<sup>-1</sup> in less productive soils. In the beginning of flowering, plants grown in productive soils were by 7.6 lower compared to those grown in less productive soils.

3. Lithuanian cultivars could be grown in more acid soil if pH<sub>KCl</sub> is not lower than 4.5 and the amount of mobile

aluminium is not higher than 25.0 mg kg<sup>-1</sup>.

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