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DISEASE REACTION OF SEVERAL LITHUANIAN AND ROMANIAN ALFALFA CULTIVARS

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ABSTRACT

The goal of the research was the evaluation of several alfalfa genetic materials for resistance to diseases under field conditions in order to identify resistant material, which can used for the development of new cultivars. Research was conducted on 10 Romanian and 10 Lithuanian alfalfa accessions. These were grown as seed crop under natural infection pressure, in Lithuania at the Institute of Agriculture, during the period 2009-2011. Three diseases were included in the study: spring black stem and leaf spots (SBSLS), downy mildew and Sclerotinia crown and stem rot (SCSR). These diseases are major factors limiting forage and seed yield of alfalfa in the wet and cool areas. The main trait differencing the accessions was the complex resistance to fungal diseases. Agro-morphological traits could not be well characterized, as they were strongly affected by diseases. Regarding resistance to spring black stem and leaf spots, the Romanian cultivars were more resistant then Lithuanian one. Larger differences were registered in the first year of vegetation, thus, average maximal SBSLS severities of Lithuanian accessions were 54 with 46, 49.5 and 65.4, whereas Romanian accessions showed an average (2009-2011) of disease severity of 45, with 17.3, 53 and 65.8% in 2009, 2010 and 2011, respectively. An average AUDPC value of Lithuanian accessions was 1958, whereas for Romanian accessions AUDPC value was 1722. Although downy mildew is less harmful then SBSLS, it can produce high losses in the genotypes which were developed in dry areas. In this experiment, severity of the disease was higher in Romanian germoplasm then in Lithuanian one. Average maximal downy mildew severities of Lithuanian accessions were between 6.0 and 15.6, whereas Romanian accessions showed disease severity between 26.9 and 36.7. On average, AUDPC values of Lithuanian accessions were between 130 and 257, whereas for Romanian accessions AUDPC values were between 448 and 668. These two diseases completely eliminated seed yield in all accessions, in 2010 and strongly negatively decreased it in 2011. Only the third growing year considerably differentiated accessions of both countries, in resistance to SCSR. These results show that Lithuanian climate is highly favourable for disease resistance screening, and identify several cultivars with better resistance, which can be recommended in alfalfa breeding.

Key words: alfalfa cultivar, resistance to diseases, *Phoma medicaginis, Peronospora trifoliorum*, *Sclerotinia trifoliorum.*

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is widely grown over the world as a perennial forage crop, due to its good quality and high herbage yield. This specie presents large diversity for various traits, since it is cultivated in contrasting environments (Julier et al., 2000).

Diseases have become one of the major factors limiting effective use of alfalfa in the wet and cool areas. Fungal diseases are the main reason decreasing forage and specially seed yield, as well as considerably reducing crop use period. Spring black stem and leaf spots (*Phoma medicaginis* var. *medicaginis* Malbr. & Roum) is one of the most harmful diseases worldwide (Liatukiene, 2012). Downy mildew (*Peronospora trifoliorum* de Bary) is less harmful. Many alfalfa cultivars are more or less susceptible to crown and root rots such as Sclerotinia stem and crown rot (*Sclerotinia trifoliorum* Eriks.). These problems can be effectively solved through the development of resistant cultivars.

Alfalfa is one of the most yielding legume grasses in Lithuania, but growing area compose small share among total area of

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grasses. The main problem for area expanding is high seed price due to very low seed yields. As a consequence, the red clover area is several folds higher than alfalfa area (Anonymous, 2011). The majority of seeds are produced abroad. The recent studies of Liatukienė et al. (2010, 2011) showed that, under Lithuanian conditions, the main constraint for stable and high seed and herbage yields is fungal diseases. Fungicides efficiency in alfalfa has not been investigated in Lithuania.

The recent trend of increasing prices for fertilizers, especially nitrogen, will force to increase cultivation area of forage legumes. Solving one of the most important alfalfa cultivation constraint should contribute to considerable increase of its growing acreage. Contrarily, effective expanding of growing area is not reliable as forages growing compete with cereal and seed-rape growing.

Comprehensive investigations of alfalfa resistance to fungal diseases showed that material of different origin were considerably different by resistance to Sclerotinia crown and stem rot (*Sclerotinia trifoliorum*) (Pierson et al., 1997; Kanbe et al., 2002), downy mildew (*Peronospora trifoliorum*) (Jie et al., 2000; Yaege and Stuteville, 2000), spring black stem and leaf spot (*Phoma medicaginis*) (Ellwood et al., 2006; Castell-Miller et al., 2007). The mentioned diseases are the main fungal diseases of alfalfa in Lithuania. Availability of genetically diverse alfalfa material is a prerequisite for developing alfalfa cultivars with improved disease resistance. Study of Lamb et al. (2006) showed that yielding improvement in cultivars which were released during 50 years period was very environment depending. The main advantage of new cultivars was multiple disease resistance, while the gain in forage yields improvement was only 0.1-0.2% per year.

The Romanian alfalfa cultivars were selected for comparison with Lithuanian ones due to intensive breeding which has been done under harsh climate and soil conditions. Also, alfalfa is widely grown in Romania, as it covers about 30% area of cultivated forage crops (Moga and Schitea, 2005; Schitea et al., 2010). The aim of this work was characterization of selected Romanian and Lithuanian alfalfa material for multi-disease resistance and some agro morphological traits in the conditions of Lithuania.

MATERIAL AND METHODS

Research was conducted at the Institute of Agriculture of Research Centre for Agriculture and Forestry, in the field of a six-years crop rotation of forage grasses in experimental years 2009-2011. The soil of the experimental site is Endocalcari-Endohypogleyic Cambisol CMg-n-w-can (pH – 7.2-7.3, P_2O_5 – 201-270 mg kg^{-1} and K_2O – 101-175 mg kg^{-1}, humus – 2.0-2.46%). Nursery was maintained under natural infection pressure. Alfalfa was sown after a black fallow without a cover crop in the first decade of July. $P_{60}K_{90}$ was applied pre-sowing one time. Every entry was sown at a rate 0.2 g per 1 meter in two 5-metre long rows in three replications, with a special hand-sowing machine Wintersteiger. The distance between the rows of a line was 0.5 m; the distance between different lines was 1.0 m. The nursery was used as a seed crop. Origin and number of tested accessions is presented in Table 1. Ten Romanian and ten Lithuanian accessions were selected for comparison. The plots were sprayed with a mix of herbicide Basagran 480 (2 l ha^{-1}) and insecticide Karate Zeon 5 CS (0.2 l ha^{-1}) when alfalfa reached the height of 10 cm in 2009. The herbicide Fenix SC 600 (3 l ha^{-1}) was applied in spring after resumption of vegetation in 2010 and 2011.

Diseases and morphological traits were evaluated in 2009-2011. Pod setting was evaluated in 2010 and 2011. Agro-morphological traits were evaluated in scores, using 1-5 score scale, where 1 is the lowest value. Plant height was evaluated in cm. Sclerotinia crown and stem rot (SCSR) were evaluated in scores using score scale 1-9, where 1 is very resistant, and 9 - very susceptible. Severity of above ground diseases was evaluated during all seasons in % using the scale: 0, 0.1, 1, 5, 10, 20, 40, 60, ≥ 80 %. The area under the disease progress curve

(AUDPC) was calculated as the total area under the graph of disease severity against time, from the first scoring to the last.

$$AUDPC = \sum_{i=1}^{n-1} [(t_{i+1} - t_i)(y_i + y_{i+1})/2],$$

where "t" is time of each reading in days, "y" is the percentage of affected foliage at each reading and "n" is the number of readings (Campbell, Madden, 1990). Statistical calculations were done using ANOVA.

Table 1. Origin and registration year of investigated cultivars

Cultivar /line	Country of origin	Registration year	Cultivar	Country of origin	Registration year
Antanė	LT	2008	Alina	RO	2001
Augūnė II	LT	1962	Adin	RO	2006
Birutė	LT	1998	Daniela	RO	2000
Malvina	LT	2006	Catinka	RO	2006
Žydrūnė	LT	1986	Cosmina	RO	2004
LIA2104	LT	Breeding population	Luxin (PI573135)	RO	1973
LIA1176	LT	Breeding population	Magnat	RO	1996
LIA1950	LT	Breeding population	Madalina	RO	2002
LIA1978	LT	Breeding population	Sandra	RO	2003
LIA2095	LT	Breeding population	Sigma	RO	1995

Table 2. Precipitations and temperature in 2009-2011 (Lithuania, Akademija weather station)

Month	Precipitations, mm				Temperature, ^{0}C			
	2009	2010	2011	1924-2011	2009	2010	2011	1924-2011
January	41.0	18.6	39.4	30.2	-2.8	-10.8	-3.2	-4.8
February	18.7	36.9	18.8	25.3	-3.5	-4.3	-7.8	-4.5
March	53.9	22.1	9.5	28.5	0.9	0.0	0.0	-0.8
April	13.1	44.2	15.6	36.9	8.9	7.3	8.8	5.8
May	26.7	94.2	46.8	52.0	12.7	13.7	13.0	12.3
June	168.6	72.4	44.3	62.4	14.6	16.2	18.1	15.7
July	90.0	142.0	115.0	73.4	18.1	21.7	19.7	17.7
August	67.1	71.1	103.8	73.7	16.8	19.8	17.4	16.7
September	48.2	52.1	54.0	51.0	13.9	11.9	13.7	12.0
October	95.4	38.0	23.9	50.2	5.2	5.0	7.6	6.8
November	63.5	71.1	21.7	44.3	3.9	3.2	3.9	1.8
December	49.9	59.6	36.2	37.2	-2.5	-7.5	1.9	-2.3
Sum/average	736.1	722.3	529.0	565.1	7.2	6.4	7.8	6.4

Weather conditions during the experimental period are presented in Table 2. Rains were very abundant in 2009; alfalfa crop establishment was very even and vigorous.

All three years had more than usually precipitations during vegetation period. This was very favourable for disease development. January was very cold with thin snow cover in 2010. Nonetheless, alfalfa overwintering was very good. Overwintering was low in some accessions in 2011, due to heavy snow cover which favoured *Sclerotinia* crown and stem rot. Conditions for pollination in 2010-2011 were favourable as nursery was intensively visited by honeybees from closely located apiaries during flowering of alfalfa.

RESULTS

The data presented in Table 3 show that statistically significant and considerable differences were observed for Spring black stem and leaf spots (SBSLS) resistance among tested accessions in 2009 (Table 3). Romanian accessions were damaged about three folds less than Lithuanian ones. Their SBSLS reaction ranged from 10.0 (Catinca, Luxin) to 25.0% (Daniela, Madalina, Sigma), whereas Lithuanian accessions were diseased from 35.0 (LIA2104) to 60.0% (LIA1176, LIA1950). The results of 2010 and 2011 showed that accessions of the both countries possessed similar resistance levels. The lowest AUDPC value (2323) was observed in Lithuanian accessions Antanė and LIA1176 and Romanian accessions with lowest AUDPC value (2478) were Magnat and Madalina. However, AUDPC values of these accessions were very similar to average values. DS in 2011 showed that resistance to SBSLS was almost identical in all accessions. The most resistant Romanian cultivars had AUDPC value 2150 and 2168 (Catinca and Madalina), whereas the most resistant Lithuanian ones showed higher AUDPC values of 2539 and 2628 (Augūnė II and Antanė).

Table 3. Spring black stem and leaf spots (*Phoma medicaginis*) resistance of investigated cultivars, during 2009-2011

Cultivar/line	2009		2010		2011		Average	
	Max DS (%)	AUDPC	Max DS (%)	AUDPC	Max DS (%)	AUDPC	Max DS (%)	AUDPC
Catinca	10.0	151	55	2544	66	2150	44	1615
Magnat	17.5	259	50	2478	66	2216	45	1651
Madalina	25.0	392	50	2478	66	2168	47	1679
Sigma	20.5	306	50	2497	66	2290	46	1698
Sandra	17.5	300	50	2497	66	2339	45	1712
Adin	12.5	193	55	2544	66	2473	45	1737
Luxin	10.0	238	55	2571	64	2463	43	1757
Alina	17.5	335	55	2497	66	2473	46	1768
Cosmina	12.5	193	55	2704	66	2473	45	1790
Augūnė II	45.0	513	45	2338	64	2539	51	1797
Antanė	40.0	444	45	2323	64	2628	50	1798
Daniela	25.0	392	55	2838	66	2216	49	1815
Žydrūnė	45.0	492	45	2396	64	2629	51	1839
Birutė	45.0	467	55	2628	64	2629	55	1908
Malvina	45.0	623	55	2628	64	2629	55	1960
LIA2095	40.0	647	45	2444	68	2991	51	2027
LIA2104	35.0	609	50	2492	68	2991	51	2031
LIA1176	60.0	827	45	2323	66	3005	57	2052
LIA1978	45.0	612	55	2699	66	2928	55	2080
LIA1950	60.0	767	55	2554	66	2946	60	2089
Average	31.4	438	51	2524	66	2559	49	1840
LSD 0.05	*1.20*	*31*	*5*	*227*	*7*	*256*	4	171
LSD 0.01	*2.60*	*66*	*9*	*454*	*14*	*537*	9	352

The data presented in Table 4 show significant differences between alfalfa genotypes for downy mildew in each of the 3 years of experiment. Average DS of Lithuanian accessions were 16.8, 7.4 and 2.1 whereas for Romanian accessions DS were 65.5, 20.0 and 6.4% in 2009, 2010 and 2011, respectively. The highest differentiation of accessions by resistance was observed in 2009, when Lithuanian accessions were diseased from 8.0 to 35.0% and Romanian ones were diseased from 55.0 to 80.0%. The most resistant Lithuanian accessions were Antanė (DS 8.0% and AUDPC value 159), Birutė, Žydrūnė and LIA1176 with DS 10.0 and AUDPC values 178, 178 and 120, respectively. The most resistant Romanian accessions were Sigma with DS 55.0 and AUDPC values 881, Magnat, Madalina and Sandra with DS 60.0 and AUDPC values 775, 885, 742, respectively. These accessions considerably exceeded the rest Romanian cultivars, but they had insufficient resistance compared to Lithuanian alfalfa material. The next two seasons were not very favourable for downy mildew resistance evaluation, as it was partially out competed by SBSLS.

DS of Lithuanian accessions decreased more than twice in 2010, but AUDPC values for some accessions, e. g. Antanė and LIA1176 increased from 159 to 218 and from 120 to 217, respectively. Such negative relationship was not observed for Romanian accessions. The mean DS of Romanian accessions decreased about three folds to 20.0% and ranged from 17.5 to 25.0%. The results of 2011 showed low differentiation of screened accessions by downy mildew severity, as DS decreased about 8 folds for Lithuanian accessions and about 10 folds for Romanian ones compared to results of 2009.

Table 4. Downy mildew resistance of Lithuanian and Romanian accessions, during 2009-2011

Cultivar/line	2009		2010		2011		Average	
	Max DS (%)	AUDPC	Max DS (%)	AUDPC	Max DS (%)	AUDPC	Max DS (%)	AUDPC
LIA1176	10.0	120	6.3	217	1.8	54	6.0	130
Antanė	8.0	159	6.3	218	1.8	48	5.4	142
Birutė	10.0	178	6.3	203	1.8	58	6.0	146
Žydrūnė	10.0	178	6.3	218	1.8	50	6.0	149
Malvina	12.0	197	6.3	203	1.8	50	6.7	150
Augūnė II	12.0	253	8.8	246	1.0	40	7.3	180
LIA1950	18.0	244	8.8	283	3.8	62	10.2	196
LIA2095	18.0	304	8.8	283	1.8	45	9.5	211
LIA1978	35.0	433	6.3	217	3.8	62	15.0	237
LIA2104	35.0	422	10.0	305	1.8	44	15.6	257
Daniela	65.0	717	20.0	520	6.3	107	30.4	448
Magnat	60.0	775	17.5	490	6.3	95	27.9	453
Cosmina	65.0	750	20.0	526	6.3	107	30.4	461
Sandra	60.0	742	17.5	588	6.3	108	27.9	479
Luxin	65.0	790	20.0	603	5.0	124	30.0	506
Adin	80.0	860	17.5	568	5.0	97	34.2	508
Madalina	60.0	885	20.0	563	8.8	98	29.6	515
Sigma	55.0	881	17.5	588	8.3	116	26.9	528
Catinca	80.0	965	25.0	663	5.0	154	36.7	594
Alina	65.0	1137	25.0	765	6.3	101	32.1	668
Average	40.8	542	13.4	402	4.2	79	19.4	341
LSD 0.05	*2.5*	*33*	*1.2*	*37*	*0.5*	*9*	1.4	26
LSD 0.01	*4.5*	*61.0*	*2.1*	*62.0*	*0.8*	*12.0*	2.5	45.0

*Maximal disease severity.

Co-development of downy mildew and SBSLS is presented in Figure 1A-C. DS at every assessment were averaged for Lithuanian and Romanian accessions groups. Downy mildew developed more intensively than SBSLS in Romanian accessions in 2009 (Figure 1A). The mean downy mildew severity reached 65.5% and only 17.3% of SBSLS severity was reached. SBSLS progressed in more downy mildew resistant Lithuanian accessions in 2009, as the mean downy mildew severity reached 16.8%, whereas SBSLS severity reached 46.0%. Development of downy mildew was clearly depressed by SBSLS in the both accessions group in 2010. The maximal downy mildew development was 8.8% in Lithuanian accessions and 25.0% in Romanian accessions, whereas SBSLS developed continuously without any depressions. The mean severity of this disease reached 55% in Lithuanian accessions and the same value, 55% in Romanian ones. SBSLS developed slightly more intensively in more downy mildew resistant Lithuanian accessions.

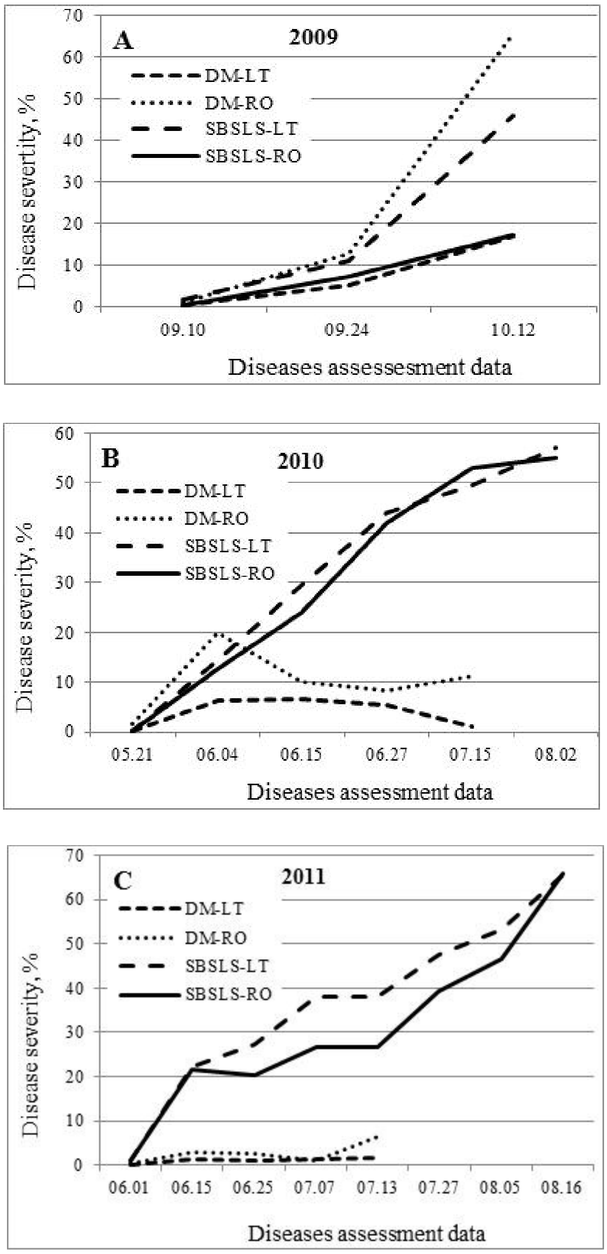

Figure 1A-C. Downy mildew (DM) and spring black stem and leaf spot (SBSLS) development in Lithuanian and Romanian cultivars and lines in 2009-2011

Development of downy mildew was weak in 2011. Dry spring clearly delayed development of SBSLS, as well as possibly depressed development of downy mildew. Downy mildew severity reached only 2.1% and 6.4% in Lithuanian and Romanian accessions, respectively. Even obscure differences in downy mildew development had considerable effect on SBSLS development in Lithuanian and Romanian accessions. SBSLS severity reached maximal difference in July, when Romanian accessions had around DS 27% and Lithuanian accession showed the mean DS around 38%.

Assessment of reaction to SCSR in 2010 showed too low disease severity for reliable differentiation of accessions (Table 5). Among Lithuanian accessions only Birutė, Malvina, Žydrūnė showed some disease damage (1.3 score). Romanian accessions showed DS from 2.0 to 2.7 scores. Only in the third growing year accessions of both countries were considerably differentiated by their resistance to SCSR. An average DS was 4.1 score for Lithuanian accessions and 6.4 score for Romanian ones.

These results showed that alfalfa SCSR resistance testing under natural infection pressure is not reliable during too short time periods. The most resistant Lithuanian accessions Malvina, LIA1176 and Augūnė II had DS 3.0, 3.0 and 3.3 scores, respectively. The least damaged Romanian accessions were Daniela, Madalina (5.7 score) and Magnat, Sandra (6.0 score). Romanian cultivars are limited source of resistance to SCSR, but some genotypes can be involved in breeding process in areas with moderate SCSR pressure.

Table 5. Sclerotinia crown and stem rot resistance of investigated cultivars in 2010-2011

Cultivar/ line	2010	2011	Cultivar/ line	2010	2011
	DS, scores			DS, scores	
Malvina	1.3	3.00	Daniela	2.0	5.70
LIA1176	1.0	3.00	Madalina	2.0	5.70
Augūnė II	1.0	3.30	Magnat	2.3	6.00
Birutė	1.3	4.00	Sandra	2.0	6.00
Žydrūnė	1.3	4.00	Alina	2.0	6.30
Antanė	1.0	4.30	Adin	2.7	6.70
LIA2095	1.0	4.30	Cosmina	2.7	6.70
LIA1950	1.0	5.00	Sigma	2.0	6.70
LIA1978	1.0	5.00	Luxin	2.0	6.80
LIA2104	1.0	5.00	Catinca	2.7	7.30
Average	1.3	4.09	Average	2.48	6.39
LSD 0.5				*0.19*	*0.86*
LSD 0.1				*0.31*	*1.31*

Statistically significant differences occurred among accessions for all agronomic traits (Table 6). The means of the traits of both groups significantly differed for plant luxuriance, leafiness, stem density, and pod setting. The herbage amount and stem density differed mainly due to lower SCSR resistance of Romanian accessions. SBSLS developed early in 2010 and pod setting was very poor in all accessions. Pod setting was higher in Lithuanian accessions, due to higher downy mildew resistance as accessions, but the results are only from one year, so it is necessary to continue this study.

Table 6. Agro-morphological traits of alfalfa during 2009-2011

Cultivar/line	Traits									
	PL	L	LS	HA	GH	SD	ST	PH1	PH	PS
Antanė	4.1	4.2	4.5	4.5	4.8	3.8	3.8	16.5	97.0	5.0
Augūnė II	4.1	4.2	4.0	4.3	4.6	3.6	3.7	15.0	94.0	5.0
Birutė	4.1	4.3	4.5	4.3	4.6	3.4	3.7	15.0	93.5	4.7
Malvina	4.3	4.3	4.4	4.5	4.8	3.8	3.7	17.5	97.5	4.7
Žydrūnė	4.0	4.2	4.5	4.6	4.8	3.5	3.8	16.5	101.0	4.7
LIA1176	4.3	4.3	4.2	4.8	4.5	3.9	3.2	15.5	99.0	4.3
LIA1950	4.3	4.3	4.2	4.5	4.5	4.0	3.7	18.0	94.0	4.3
LIA1978	3.9	4.0	3.5	4.5	4.7	3.7	3.6	17.0	94.5	4.3
LIA2095	4.1	4.2	4.2	4.2	4.7	4.0	3.8	17.5	100.5	4.3
LIA2104	4.2	4.2	4.2	4.5	4.3	3.8	4.0	15.0	94.5	4.2
Adin	3.4	3.5	3.9	2.9	4.3	2.8	3.6	16.0	93.0	3.7
Magnat	3.7	3.8	4.0	3.5	4.8	3.2	3.7	16.5	96.0	3.7
Madalina	3.7	3.8	4.0	3.7	4.8	3.4	3.4	18.5	87.5	3.7
Sigma	3.8	3.9	4.0	3.6	4.8	3.4	3.5	15.5	97.0	3.7
Alina	3.5	3.7	4.0	3.3	4.8	3.3	3.5	16.0	85.5	3.3
Catinka	3.1	3.3	3.5	2.6	4.3	2.6	3.6	16.5	87.0	3.3
Sandra	3.6	3.8	4.0	3.8	4.5	3.5	3.4	18.0	97.0	3.3
Cosmina	3.3	3.7	3.9	3.5	4.7	3.1	3.6	16.0	97.0	3.0
Daniela	3.5	3.7	4.0	3.5	4.5	3.1	3.7	17.5	94.5	3.0
Luxin	3.7	3.7	3.4	3.1	4.3	3.0	3.9	17.0	84.0	2.0
Average	3.8	4.0	4.0	3.9	4.6	3.4	3.6	16.6	94.2	3.9
LSD 0.5	*0.41*	*0.37*	*0.74*	*0.41*	*0.27*	*0.38*	*0.43*	*2.41*	*10.69*	*0.48*
LSD 0.1	*0.55*	*0.49*	*1.00*	*0.54*	*0.36*	*0.51*	*0.59*	*3.29*	*14.61*	*0.64*

PL – plant luxuriance, L – leafiness, LS – leaf size, HA – herbage amount, GH – growth habit, SD – stem density, ST – stem thickness, PH1 – plant height 2 weeks after renewal of vegetation, 2010-2011, PH – plant height at flowering, 2010-2011, PS – pod setting, 2011

The traits that were considerably affected by performance of diseases are presented in Table 7. Lower disease resistance highly negatively influenced alfalfa crop performance.

Downy mildew and SCSR severities negatively influenced all traits. Leaf size and plant height were depressed slightly or moderately (r = -0.32 and -0.59**). Plant luxuriance, leafiness, stem density, pod setting were depressed highly negatively by severities of diseases (r=-0.70 and -0.88**). Higher SBSLS severities were highly positively related with many morphological traits in 2009 and 2011 and weakly negatively in 2010. Such relations has simple reason as SBSLS developed better in more dense crop with higher air and leaves moisture.

Table 7. The relationship among the performance of diseases and other traits

Disease	Traits						
	PL	L	LS	HA	SD	PH	PS
Downy mildew 2009, AUDPC	-0.84**	-0.85**	-0.59**	-0.83**	-0.70**	-0.53*	-0.81**
Downy mildew 2010, AUDPC	-0.82**	-0.83**	-0.54**	-0.84**	-0.70**	-0.53*	-0.85**
Downy mildew 2011, AUDPC	-0.84**	-0.81**	-0.59**	-0.80**	-0.74**	-0.41*	-0.88**
Spring black stem and leaf spot 2009, AUDPC	0.82**	0.77**	0.38*	0.79**	0.81**	0.34*	0.67**
Spring black stem and leaf spot 2010, AUDPC	-0.49*	-0.50*	-0.51*	-0.48*	-0.52*	-0.41*	-0.41*
Spring black stem and leaf spot 2011, AUDPC	0.76**	0.72**	0.33*	0.73**	0.75**	0.37*	0.53*
Sclerotinia stem and crown rot, 2010, severity	-0.86**	-0.84**	-0.42*	-0.83**	-0.83**	-0.32*	-0.76**
Sclerotinia stem and crown rot, 2011, severity	-0.80**	-0.81**	-0.58*	-0.79**	-0.68*	-0.44*	-0.84**

PL – plant luxuriance, L – leafiness, LS – leaf size, HA – herbage amount, SD – stem density, PH1 PH – plant height at flowering, 2010-2011, PS – pod setting, 2011.

DISCUSSION

Development of diseases shows that Lithuanian climate is very favorable for alfalfa resistance investigations. However, this situation negatively influences alfalfa growing in Lithuania. Seed production inside country is very limited and its multiplication abroad greatly increases seed price, and this in turn decreases growing areas. Foreign alfalfa cultivars grown without previous testing are, in most cases, heavily damaged by diseases, which even more raises mistrust of farmers in alfalfa.

The resistance to SBSLS, which is one of the main disaster of seed crop alfalfa in Lithuania, was similar among accessions of both countries. Accessions were compared by maximal DS and AUDPC. Some differences were found in 2009, when period for disease development was much shorter and precipitation amount was slightly lower than in 2010 and 2011. Study of Castell-Miller et al. (2007) showed that only a period of few days after inoculation with *P. medicaginis* was suitable for detecting reliable differences between susceptible and moderately resistant genotypes. No considerable differences were seen after 10 days for lower leaves and some differences were seen for upper leaves. This shows that some relatively more resistant accessions among material with rather low resistance could be found using screening under moderately favorable conditions for disease development. However, our field situation showed that both Lithuanian and Romanian accessions possessed too low resistance for pod setting under severe infection pressure in 2010. Annual medics, which were more resistant than alfalfa (O'Neill and Bauchan, 2003), could serve as sources of resistance to SBSLS. Also, the study of Kamphuis et al. (2008) revealed two recessive quantitative trait loci in *M. truncatula*. However, this approach of resistance breeding would last long enough. On the other hand, if no reliable resistance sources exist among alfalfa, even a long approach can be acceptable. Up to now, comprehensive mapping of SBSLS resistance QTL's in alfalfa was not done. Therefore, it might be possible to find some alfalfa accessions with diverse and slightly more efficient QTLs than that in the rest of accessions. But, if efficiency of these QTLs would appear low in any way, their concentration in new genotypes could last as long as introduction of more efficient QTLs from medic species.

Downy mildew was very harmful disease to susceptible accessions tested. One of disease peculiarities is that causal agent overwinters in plants and starts to develop and spread after resumption of vegetation (Leath et al.. 1998). Usually it damages top of plants. When infection is severe, very susceptible plants can be destroyed completely just after regrowth in spring. Top damage of plants made heavy damage to seed production even under relatively low disease severity in 2010 and 2011, in all accessions. Seeds were not produced in 2010 at all, due to higher downy mildew development. Resistance to this disease depends on combinations of mono- and polygenes. Since alfalfa is cross-pollinating plant, it populations consists of plants which vary by resistance (Skinner and Stuteville. 1985, 1989). Disease agent can adapt to monogenes very rapidly. Therefore, a more promising resistance breeding strategy should rely on accumulation of polygenes (Keller et al.. 2000). Investigations of alfalfa accessions with diverse geographical origin showed that accessions originating from different regions were more susceptible. More resistant accessions usually originate from regions with similar climate (Skinner and Stuteville. 1992; Yaege and Stuteville. 2000). Skinner and Stuteville (1992) did not find immune to downy mildew accession among several hundreds of alfalfa accessions. Romanian accessions could hardly improve downy mildew resistance of Lithuanian alfalfa breeding material. The relatively low downy mildew resistance level in Romanian accessions was determined by the environmental conditions, which were not favourable to disease development at the same level in Lithuania.

Differentiation of tested accessions by SCSR resistance was considerable and ranged from resistant to susceptible. It shows that crops of non-adapted cultivars of alfalfa under Lithuanian conditions can be heavily thinned in the second exploitation year only by one disease SCSR under natural disease pressure. It seems that resistance donors should be selected from countries with similar conditions for disease development. Among three diseases, SCSR required the longest time for accessions differentiation under natural infection pressure. Only the third year showed satisfactory results. It seems that SCSR resistance breeding under natural field conditions is long lasting, as it was revealed in many publications. SCSR resistance breeding has been slow, because breeders tried to select resistant population, without paying attention to construction of such populations from individuals with better resistance. Usually, the most perspective resistance breeding was carried on under field conditions with artificial infection (Pierson et al.. 1997; Kanbe et al.. 2002). Many improved methods were developed for selection of resistant individuals (Rowe, 1993; Pratt. 1996; Halimi et al., 1998). However, wide exploitation of these methods in breeding programs was not reflected in literature.

CONCLUSIONS

The main trait differentiating the accessions was the complex resistance to fungal diseases. Accessions from Romania possessed higher resistance in spring black stem and leaf spot and Lithuanian accessions possessed higher resistance to downy mildew and Sclerotinia crown and stem rot. Romanian cultivars Catinca, Madalina and Magnat can be used as sources to improve resistance for spring black stem and leaf spot in breeding material. Lithuanian cultivars Antanė, Birutė and Žydrūnė can be used for downy mildew resistance breeding, whereas Augūnė II and Malvina can provide Sclerotinia crown and stem rot resistance. Sclerotinia crown and stem rot highly negatively influenced herbage amount in susceptible accessions. Spring black stem and leaf spot and downy mildew highly negatively decreased pod setting and later seed development.

REFERENCES

Anonymous. Statistical Department of Lithuania, 2011. *Agriculture, hunting, forestry and fishery*. http://db1.stat.gov.lt

Campell, C.L., Madden, L,V., 1990. *Introduction to Plant Disease Epidemiology*. John Wiley & Sons, New York.

Castell-Miller, C.V., Zeyern, R.J., Samac, D.A., 2007. *Infection and development of Phoma medicaginis on moderately resistant and susceptible alfalfa genotypes*. Can. J. Plant Pathol., 29: 290-298.

Ellwood, S.R., Kamphuis, L.G., Oliver, R.P., 2006. *Identification of sources of resistance to Phoma medicaginis isolates in Medicago truncatula SARDI core collection accessions, and multigene differentiation of isolates*. Phytopathology, 96: 1330-1336.

Halimi, E.S., Rowe, D.E., Pratt, R.G., 1998. *Responses of alfalfa to stem-tip inoculations with five isolates* *of Sclerotinia trifoliorum*. Crop Sci., 38: 1179-1182.

Jie, L.C., Biao, N.Z., Wen, W.Y., Wang, Y.R., 2000. *Evaluation for alfalfa germplasm resistance to downy mildew under alpine grassland conditions*. Acta Prataculture Sinica, 9: 44-51.

Julier, B.. Huyghe, C., Ecalle, C., 2000. *Within and among cultivar genetic variation in alfalfa: forage quality, morphology and yield*. Crop Sci., 40: 365-369.

Kamphuis, L.G., Lichtenzveig, J., Oliver, R.P., Ellwood, S.R., 2008. *Two alternative recessive quantitative trait loci influence resistance to spring black stem and leaf spot in Medicago truncatula*, BMC Plant Biol. DOI: 10.1186/1471-2229-8-30.

Kanbe, M., Mizukami, Y., Fujimoto, F., 2002. *Improvement of resistance to Sclerotinia crown and stem rot of alfalfa through phenotypic recurrent selection*. Jpn. Agr. Res. Q., 36: 1-5.

Keller, B., Feuillet, C,, Messmer, M.. 2000. *Genetics of disease resistance*. In: Slusarenko, A.J., Fraser, R.S.S., van Loon, L.C. (eds.), Mechanisms of Resistance to Plant Diseases. Kluwer Academic Publishers, Dordrecht: 101-160.

Leath, K.T., Erwin, D.C., Griffin, G.D., 1998. *Diseases and nematodes*. In: Hanson, A.A. (ed.), Alfalfa and alfalfa improvement. American Society of Agronomy, Madison: 621-670.

Liatukienė, A., Liatukas, Ž., Ruzgas, V., 2010. *Reaction of lucerne (Medicago spp.) to fungal diseases caused by Phoma medicaginis*. Agric. Sci., 17: 25-31.

Liatukienė, A., Liatukas, Ž., Ruzgas, V., 2011. *Development of downy mildew and spring black stem and leaf spot in lucerne seed crop.* Agric. Sci., 18: 95-99.

Aurelija Liatukienė, 2012. *Investigation of genetic diversity of lucerne (Medicago spp.) by identifying resistance to pathogens and mobile aluminium,* Summary of doctoral dissertation Agricultural sciences, agronomy, Aleksandras Stulginskis University Lithuanian Research Centre For Agriculture And Forestry.

Moga, I., Schitea, M., 2005. *Tehnologii mederne de producere a semintelor la plantele furajere*. Ed. Ceres, Bucuresti: 23-54.

O'Neill, N.R., Bauchan, G. R., 2003. *Reactions in the annual Medicago spp. core germplasm collection to Phoma medicaginis*. Plant Dis., 87: 557-562.

Pierson, P.E., Martin, S.K., Rhodes, L.H., 1997. *Selection for resistance to Sclerotinia crown and stem rot in alfalfa*. Crop Sci., 37: 1071-1078.

Pratt, R.G., 1996. *Screening for resistance Sclerotinia trifoliorum in alfalfa by inoculation of excised leaf tissue*. Phytopathology, 86: 923-928.

Rowe, D.E., 1993. *Oxalic acid effects in exudates of Sclerotinia trifoliorum and S. sclerotiorum and potential use in selection*. Crop Sci., 33: 1146-1149.

Skinner, D.Z., Stuteville, D.L., 1992. *Geographical variation in alfalfa accessions for resistance to two isolates of Peronospora trifoliorum.* Crop Sci., 32: 1467-1470.

Skinner, D.Z., Stuteville, D.L., 1988. *Polygenes in diploid alfalfa that affect resistance to downy mildew*. Crop Sci., 28: 508-511.

Skinner, D.Z., Stuteville, D.L., 1985. *Quantitatively inherited reactions of alfalfa to Peronospora trifoliorum*. Phytopathology, 75: 717-721.

Schitea, M., Varga, P., Martura, T., Petcu, E., Drãgan, L., Oprea, G., Dihoru, A., 2010. *Results in alfalfa breeding at NARDI Fundulea.* Proceeding of the XII International Symposium on Forage Crops of Republic of Serbia, 26: 117-126.

Yaege, J.R., Stuteville, D.L., 2000. *Reactions in the annual Medicago core germplasm collection to two isolates of Peronospora trifoliorum from alfalfa*. Plant Dis., 84: 521-524.