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REACTION OF THE LITHUANIAN ALFALFA BREEDING POPULATIONS TO *PHOMA MEDICAGINIS* UNDER COOL TEMPERATE CLIMATE CONDITIONS

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Abstract

The alfalfa *Phoma medicaginis* resistance was evaluated on 100 accessions with different development date. Weather conditions favoured high disease pressure and differentiation of tested accessions was not clear in 2011. Accessions were compared by maximal disease severity (DS) and area under disease progress curve (AUDPC). DS ranged from 10 to 60% and AUDPC value ranged 119–727 in 2009. DS and AUDPC values were higher in 2010 and 2011, DS ranged 40–65 and 66–68% and AUDPC ranged 2263–2928 and 2853–3006. Development date of accessions showed low impact on resistance. The correlations between DS and AUDPC results of accessions across years highly varied (r=–0.189 – 0.828**) due to low differentiation of accessions resistance. Selection of alfalfa material promising by *Phoma medicaginis* resistance should be done under conditions moderately favourable for development of this disease.

Key words: *Medicago sativa*, Resistance, Foliar diseases.

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. It has high advantage over the rest forages grasses under dry and warm climate. This species presents large diversity for various traits since it's cultivated in contrasting environments (Julier *et al*., 2000). The recent trend of increasing prices for fertilizers, especially nitrogen as well as rising area of organic farming will force to increase cultivation area of forage legumes. However, deficiency of high complex disease resistance is one of the main constrains for successful cultivation of alfalfa durable crop (Lamb *et al*., 2006).

Alfalfa is one of the most yielding perennial forage legume grasses in Europe (Mosimnan & Lehmann, 2002) and Lithuania also (Šlepetys, 2008), it also has high potential for biogas production (Nekrošius *et al*., 2014) but growing area compose fractional share among total area of forages in Lithuania (Anon., 2012b). The recent investigation of alfalfa disease resistance in Lithuania showed that the broad range of diseases can heavily damage alfalfa in Lithuania and the highest negative impact diseases make on the seed yield (Liatukienė, 2012). It is the soundest reason why alfalfa area is so insignificant in Lithuania and neighbouring countries of the Baltic Sea region (Anon., 2012a). Under dry hot climate conditions alfalfa produces high seed yields (Rashidi *et al*., 2009) and this crop is dominant forage source (Hanson, 1998).

Spring black stem and leaf spot disease caused by *Phoma medicaginis* is one of the harmful fungal diseases of alfalfa in temperate and Mediterranean regions (Rodriguez *et al*., 1990; Nutter *et al*., 2002). It has tendency to spreading to the new areas in the North America (Akamatsu *et al*., 2008; Wunsch *et al*., 2010). This disease is extremely harmful under wet cool temperate climate of Lithuania as it alone can destroy all seed yield (Liatukienė, 2012). Limited studies concerning *Medicago* spp. resistance to *Phoma* spp. revealed some possibilities to improve resistance. *Medicago sativa* showed variable reaction of accessions (Wang *et al*., 2004; Castell-Miller *et al*., 2007). However, much comprehensive and wider researches were conducted with model species *M. truncatula* (Ellwood *et al*., 2006) and annual species (O'Neill & Bauchan, 2003). It was revealed that *M. truncatula* resistance to *P. medicaginis* depends on quantitative trait locus (Kamphuis *et al*., 2008). This research showed that available *Medicago* material is not sufficiently resistant, but improvements can be done developing more resistant material.

Information about alfalfa cultivars resistance to spring black stem and leaf spot in Europe is scant. Comprehensive recent research including considerable number of accessions was not found. Therefore, the present study aimed to determine the *Phoma medicaginis* resistance of alfalfa accessions of different development date under cool temperate climate conditions of Lithuania.

Material and Methods

Plant material and field design: Research was conducted at the Institute of Agriculture of Research Centre for Agriculture and Forestry in the field of a six-course 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 per kg and K_2O – 101-175 mg per kg of soil, 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 in 2009. The complex phosphorus and potassium fertiliser was applied once before sowing at the rate $P_{60}K_{90}$. Every accession was sown at a rate 0.2 g scarified seed per 1 meter in two 5-metre long rows in three replications with special hand-sowing machine "Plotmatic 1R", produced by Wintersteiger, Austria. 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. The experimental material composed of 100 accessions of alfalfa of different development date (Table 2). The plots were sprayed with mix of herbicide Basagran 480 (2 l ha^{-1}) (active ingredient bentazon 480 g l^{-1}) and insecticide Karate Zeon 5 CS (0.2 l ha^{-1}) (active ingredient lambda-cihalotrin 50 g l^{-1}) when alfalfa after germination reached the height of 10 cm in 2009. The herbicide Fenix SC 600 (3 l ha^{-1}) (active ingredient aklonifen 600 g $^{l-1}$) was applied in spring after resumption of vegetation in 2010 and 2011. The insecticide Karate Zeon 5 CS was applied when pests became harmful in 2010 and 2011.

Evaluation of resistance: Spring black stem and leaf spot was evaluated in 2009-2011. Disease severity (DS) was

evaluated during all season in percents using the scale: 0, 0.1, 1, 5, 10, 20, 40, 60, and 80% (Campbell & Madden, 1990).

Weather conditions: Weather conditions during experimental period are presented in Table 1. Rains were very abundant in 2009; alfalfa crop establishment was very even and vigorous. All three years had more than usual precipitations during vegetation period. It was very favourable for disease development. Winter was very cold with weak snow cover in 2010. Nonetheless, alfalfa overwintering was very good. Overwintering was weak in some accessions in 2011 due to heavy show cover that favoured development of Sclerotinia crown and root rot (*Sclerotinia trifoliorum*).

Statistical analysis: 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 in days of each reading, "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.

Results

Development of spring black stem and leaf spot: The disease started to develop when alfalfa reached budding stage in all years when rows were closed and foliage remained wet longer. The disease was suppressed by downy mildew in 2009, when soil did not contain infected plant residues and pathogen spores came from neighbouring fields. However, it was by far the dominant disease in 2010–2011. Fig. 1 shows the spring black stem and leaf spot development on three alfalfa genotypes differing in AUDPC values in 2009–2011.

The maximal disease severities were rather similar among years. Whereas AUDPC values were the lowest in 2009 due to short disease development period and the highest in 2011 due to longer diseases development period.

AUDPC values among the most resistant and susceptible alfalfa genotype differed 2.3-fold from 321 to 726 in 2009. This difference was only 1.1 and 1.03 fold from 2421 to 2703 and from 2856 to 2946 in 2010 and 2011, respectively. Maximal disease severity among alfalfa genotypes considerably differed only in 2009, when genotypes differentiation was highest. Genotypes presented in Fig. 1 were damaged from 17.5 to 55%. Whereas, the highest maximal disease severity was in 2011 when it ranged from 66 to 68%. It was similar in 2010 when it ranged from 50 to 65%.

Severe spring black stem and leaf spot development during all experimental years shows excellent possibility to test alfalfa resistance in relatively short terms. Also, it shows high aggressiveness of disease and its potential of harmfulness. Low disease resistance shows sound impact for development of the more resistant cultivars and use the less susceptible ones.

Alfalfa of spring black stem and leaf spot reaction: The accessions presented in Table 2 are sorted in ascending order of maximal disease severity in 2009. The alfalfa accessions differed considerably by disease severities and AUDPC values in 2009 when these ranged from 10 to 60% and from 239 to 818, and reflected rather optimal differentiation of resistance. However, differentiation of accession was low in 2010 and 2011 due to high disease pressure. DS and AUDPC values differed about 6 and 3.4 folds in 2009, whereas these traits differed about 1.6 and 1.3 fold in 2010 and only 1.03 and 1.05 fold in 2011. The differences between DS and AUPDC were similar. However, accessions with the same DS had different AUDPC values in all years. For example, accessions diseased up to 20% and 40% had AUDPC values from 263 to 485 and from 424 to 668 in 2009. Similar situation was in 2010 when accession diseased up to 50% and 65% had AUDPC values from 2265 to 2728 and from 2588 to 2928, respectively. However, such relationship was not found in 2011. AUDPC values showed additional possibility to differentiate alfalfa accessions by resistance when disease severity was the same. However, calculation of AUDPC values can be done only after several DS assessments. On the other hand, spring black stem and leaf spot development (Fig. 1) in 2009–2011 showed necessity to evaluate disease development as longer as possible. Overview of the disease development during all season, together comparing accessions by calculated AUDPC values allows selection of accessions possessing the highest resistance.

Table 1. Precipitations and temperature (Lithuania, Akademija weather station)

Month	Precipitations, mm				Temperature, °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

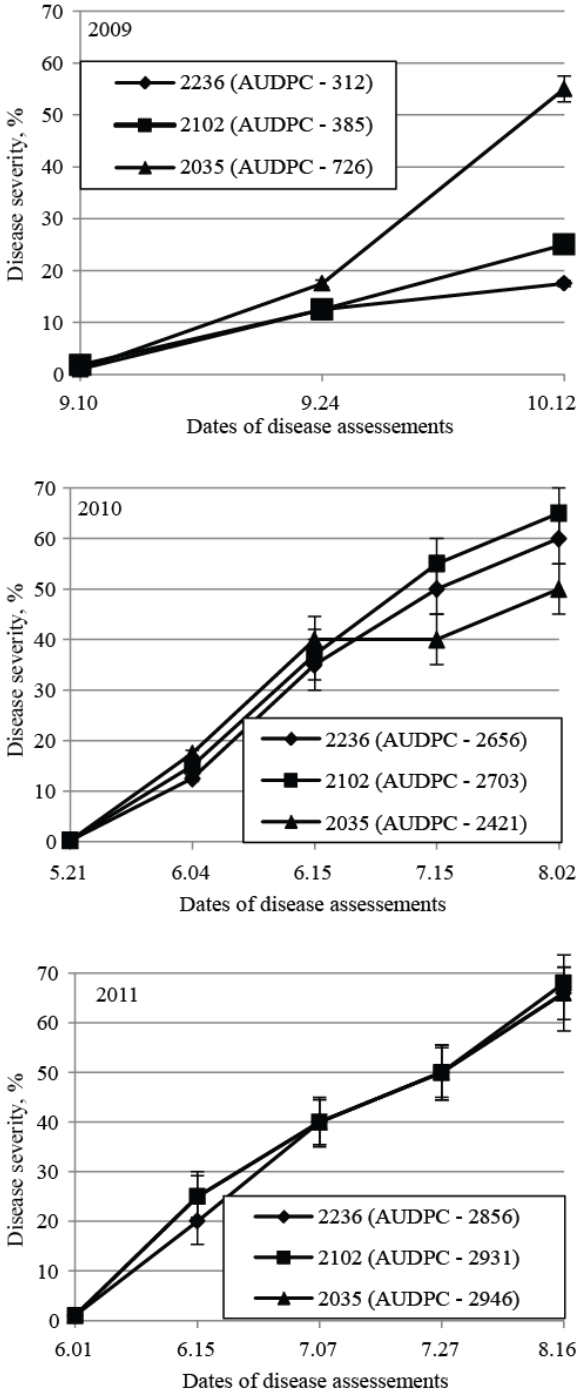

Fig. 1. Development of foliar disease caused by *Phoma medicaginis* in alfalfa accessions with different AUDPC values

Development data of the host accessions showed low impact on resistance. Only AUDPC values correlated with accessions development data weakly in 2011 (0.413*). The correlation coefficients between DS and AUDPC across years highly varied (r=–0.189 – 0.828**) (Table 3). DS strongly correlated with AUDPC values (r=0.828** and 0.827**) in 2009 and 2010. AUDPC values showed weak correlation (r=0.336*) between 2009 and 2010 years. The rest relationships showed too low correlation level for consideration.

Discussion

Development and severity of spring black stem and leaf spot shows that wet and cool Lithuanian climate is very favourable for alfalfa resistance investigations. However, cultivars differentiation was insufficient due to too low resistance and very high disease pressure. This situation highly negatively influences alfalfa growing in Lithuania and the rest countries with similar climate. Seed production inside country is very limited (Anon., 2012b) 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.

Spring black stem and leaf spot was very harmful disease to all accessions in 2010 and 2011. One of disease peculiarities is that causal agent overwinters in plant residues and soil and starts to spread after resumption of vegetation but considerable disease symptoms are visible only after months or later due to slow disease development (Hanson., 1998; Castell-Miller *et al*., 2007). Disease development can be delayed by insufficient precipitations but under our conditions it depressed disease only until row closure in 2011. At that moment plants stand become dense enough to maintain lower leaves wet longer time that favoured infection and disease development. The disease starts to destroy plants from lower levels but at pod setting period it can cover all plant that occurred in 2010 when pods and seeds were destroyed. 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. Whereas, the gain in forage yields improvement was only 0.1-0.2% per year. It shows that conventional breeding based on field evaluations and selections makes SBSLS resistance breeding progress to slow for development of considerably improved cultivars possessing acceptable resistance level under heavy disease pressure.

Resistance to this disease depends on polygenes. Since alfalfa is cross pollinating plant, its populations consist of plants which vary by resistance (Ellwood *et al*., 2006, Kamphuis *et al*., 2008). It shows possibility to develop more resistant cultivars but initially selection of more resistant plants should be done under greenhouse or laboratory conditions that substantially differentiate cultivars and plants by SBSLS resistance (O'Neill & Bauchan 2003; Wang *et al*., 2004; Barbetti, 2007; Castell-Miller *et al*., 2007). Greenhouse growing technology requires very high inputs. Development of the new the populations with considerably higher resistance level takes several selection cycles which can continue up to 10 and more years (Kanbe *et al*., 2002). Development period of few decades shows that only some critical stages could be performed under controlled conditions.

Table 2. Maximal severities and AUDPC values of foliar disease caused by *Phoma medicaginis* in alfalfa accessions.

Alfalfa accession	Development dates	Maximal disease severity, %			AUDPC value		
		2009	2010	2011	2009	2010	2011
2450	2003	10 a*	65 bc	66 a	242 a	2702 def	2979 cde
2446	2003	12.5 ab	60 b	66 a	239 a	2577 cde	2979 cde
2448	2003	12.5 ab	65 bc	66 a	354 abc	2759 ef	2979 cde
2250	1992	12.5 ab	65 bc	66 a	411 bcd	2769 ef	2979 cde
2454	2004	17.5 ab	50 ab	66 a	371 bc	2269 a	2979 vde
2251	1992	17.5 ab	65 bc	66 a	373 bc	2766 ef	2979 cde
2152	1988	17.5 ab	50 ab	68 ab	269 ab	2398 bcd	2991 de
2236	1987	17.5 ab	60 b	68 ab	312 abc	2656 de	2991 de
1769	1980	20 bc	65 bc	66 a	263 a	2800 efg	2868 abc
462	1970	20 bc	40 a	66 a	339 abc	2527 cde	2919 bc
2070	1984	20 bc	60 b	68 ab	393 bc	2807 efg	2919 bc
618	1970	20 bc	50 ab	66 a	307 ab	2304 ab	2924 bc
900	1973	20 bc	50 ab	66 a	339 abc	2363 bc	2924 bc
753	1972	20 bc	50 ab	66 a	428 bcd	2421 cd	2924 bc
809	1973	20 bc	50 ab	66 a	480 cd	2421 cd	2924 bc
2125	1985	20 bc	50 ab	68 ab	396 bc	2367 bc	2931 bcd
2100	1985	20 bc	65 bc	68 ab	339 abc	2588 cde	2931 bcd
2305	1995	20 bc	40 a	66 a	396 bc	2360 bc	2979 cde
2427	2002	20 bc	60 b	66 a	485 cde	2718 def	2979 cde
282	1957	20 bc	60 b	66 a	310 ab	2776 ef	2979 cde
2200	1986	20 bc	50 ab	68 ab	399 bc	2301 ab	2991 de
2182	1986	20 bc	50 ab	68 ab	292 ab	2393 bcd	2991 de
2138	1987	20 bc	50 ab	68 ab	428 bcd	2452 cd	2991 de
2242	1986	20 bc	60 b	68 ab	292 ab	2718 def	2991 de
148	1951	25 bcd	50 ab	66 a	446 bcd	2613 de	2886 abc
160	1953	25 bcd	50 ab	66 a	355 abc	2653 de	2886 abc
961	1975	25 bcd	60 b	66 a	441 bcd	2602 de	2919 bc
638	1970	25 bcd	50 ab	66 a	385 bc	2358 bc	2924 bc
497	1970	25 bcd	60 b	66 a	356 abc	2708 def	2924 bc
1095	1974	25 bcd	65 bc	66 a	383 bc	2759 ef	2924 bcd
231	1957	25 bcd	65 bc	66 a	355 abc	2702 def	2928 bcd
309	1964	25 bcd	60 b	66 a	355 abc	2718 def	2928 bcd
1775	1980	25 bcd	60 b	66 a	360 abc	2753 ef	2928 bcd
2102	1985	25 bcd	65 bc	68 ab	385 bc	2703 def	2931 bcd
386	1971	25 bcd	60 b	66 a	383 bc	2654 de	2979 cde
771	1970	25 bcd	50 ab	66 a	354 abc	2679 de	2979 cde
1861	1981	25 bcd	60 b	66 a	655 fg	2718 def	2979 cde
2858	2005	25 bcd	60 b	66 a	478 cd	2753 ef	2979 cde
190	1954	25 bcd	60 b	66 a	473 cd	2774 ef	2979 cde
4181	2006	25 bcd	60 b	66 a	653 fg	2807 efg	2979 cde
2133	1987	25 bcd	50 ab	68 ab	473 cd	2398 bcd	2991 de
2142	1987	25 bcd	50 ab	68 ab	470 cd	2421 cd	2991 de
2145	1988	25 bcd	60 b	68 ab	383 bc	2602 de	2991 de
2187	1986	25 bcd	60 b	68 ab	475 cd	2810 efg	2991 de
1761	1978	30 cd	60 b	66 a	402 bcd	2748 def	3006 def
146	1951	35 cd	50 ab	66 a	481 cde	2728 def	2856 ab
2060	1984	35 cd	65 bc	68 ab	628 efg	2766 ef	2913 bc
901	1972	35 cd	40 a	66 a	450 bcd	2263 a	2919 bc
824	1971	35 cd	60 b	66 a	436 bcd	2634 de	2919 bc
698	1970	35 cd	60 b	66 a	541 de	2708 def	2924 bc
385	1969	35 cd	60 b	66 a	685 fgh	2717 def	2928 bcd

Table 2. (Cont'd.).

Alfalfa accession	Development dates	Maximal disease severity, %			AUDPC value		
		2009	2010	2011	2009	2010	2011
220	1956	35 cd	60 b	66 a	478 cd	2868 fg	2946 cd
2419	1998	35 cd	60 b	66 a	536 de	2722 def	2979 cde
2261	1992	35 cd	60 b	66 a	748 gh	2723 def	2979 cde
2447	2003	35 cd	65 bc	66 a	481 cde	2756 ef	2979 cde
2071	1984	35 cd	60 b	66 a	808 gh	2776 ef	2979 cde
2204	1985	35 cd	50 ab	68 ab	573 def	2367 bc	2991 de
2146	1988	35 cd	60 b	68 ab	439 bcd	2544 cde	2991 de
2104	1985	35 cd	60 b	68 ab	539 de	2717 def	2991 de
2119	1985	35 cd	60 b	68 ab	566 def	2815 efg	2991 de
1985	1982	40 d	50 ab	66 a	481 cde	2363 bc	2868 abc
343	1969	40 d	50 ab	66 a	558 de	2559 cde	2868 abc
2049	1984	40 d	50 ab	68 ab	618 ef	2394 bcd	2871 abc
2050	1984	40 d	60 b	68 ab	668 fg	2709 def	2871 abc
1986	1984	40 d	50 ab	66 a	453 cd	2367 bc	2886 abc
885	1972	40 d	60 b	66 a	497 cde	2453 cd	2919 bc
934	1975	40 d	50 ab	66 a	526 de	2363 bc	2924 bc
1087	1974	40 d	65 bc	66 a	526 de	2853 fg	2924 bcd
2067	1984	40 d	60 b	68 ab	526 de	2779 efg	2931 bcd
1772	1980	40 d	50 ab	66 a	424 bcd	2451 cd	2946 cd
1774	1980	40 d	50 ab	66 a	497 cde	2457 cd	2946 cd
2456	2005	40 d	50 ab	66 a	589 ef	2301 ab	2979 cde
631	1971	40 d	60 b	66 a	589 ef	2776 ef	2979 cde
2257	1990	40 d	65 bc	66 a	620 ef	2823 efg	2979 cde
2248	1992	40 d	60 b	68 ab	620 ef	2619 de	2991 de
2095	1985	40 d	60 b	68 ab	586 ef	2793 efg	2991 de
2064	1984	45 de	50 ab	68 ab	663 fg	2367 bc	2853 a
2041	1984	45 de	65 bc	68 ab	663 fg	2797 efg	2853 a
2068	1984	45 de	60 b	68 ab	663 fg	2749 def	2871 abc
1981	1982	45 de	60 b	66 a	573 def	2776 ef	2886 abc
2052	1984	45 de	60 b	68 ab	663 fg	2807 efg	2913 bc
2249	1992	45 de	65 bc	66 a	636 efg	2756 ef	2919 bc
1172	1975	45 de	60 b	66 a	573 def	2602 de	2928 bcd
2029	1984	45 de	60 b	66 a	573 def	2656 de	2928 bcd
1978	1982	45 de	60 b	66 a	573 def	2806 efg	2928 bcd
1941	1982	45 de	60 b	66 a	639 efg	2776 ef	2946 cd
2252	1992	45 de	65 bc	66 a	725 fgh	2822 efg	2979 cde
2188	1986	45 de	60 b	68 ab	725 fgh	2810 efg	2991 de
2051	1984	45 de	60 b	68 ab	573 def	2824 efg	2991 de
2056	1984	45 de	65 bc	68 ab	665 fg	2854 fg	2991 de
322	1967	50 de	50 ab	66 a	595 ef	2449 cd	2886 abc
2063	1984	55 ef	65 bc	68 ab	818 ghi	2858 fg	2913 bc
915	1975	55 ef	60 b	66 a	648 efg	2718 def	2928 bcd
2097	1985	55 ef	65 bc	68 ab	640 efg	2782 efg	2931 bcd
2035	1984	55 ef	50 ab	66 a	726 fgh	2421 cd	2946 cd
1754	1978	55 ef	50 ab	66 a	640 efg	2451 cd	3006 def
1750	1979	55 ef	60 b	66 a	640 efg	2823 efg	3006 def
1950	1982	60 f	65 bc	66 a	719 fgh	2872 fg	2946 cd
1970	1982	60 f	65 bc	66 a	779 gh	2928 fgh	2946 cd
1176	1975	60 f	65 bc	66 a	779 gh	2871 fg	3006 def
Average		33.2	54.5	57.5	505	2639	2947

* Means fallowed by the same letters do not differ according to Duncan's Multiple Range Test at probability $p<0.05$

Table 3. Correlation coefficients among foliar disease caused by *Phoma medicaginis* severities (DS) and AUDPC values.

Traits	2009-DS	2010-DS	2011-DS	2009-AUDPC	2010-AUDPC
2010–DS	0.171				
2011–DS	0.006	0.058			
2009–AUDPC	0.828**	0.237*	0.070*		
2010–AUDPC	0.254*	0.827**	-0.014	0.336*	
2011–AUDPC	-0.189	0.140	0.109	-0.062	0.106

*p<0.05, **<0.01

Screening populations resistant at seedling stage can denote the most resistant seedlings (Djebali, 2013). Also employment of *P. medicaginis* isolates with different aggressiveness could highlight the most resistant populations or individual plants (Castell-Miller *et al*., 2008; Djebali, 2013). Some alternatives such as bacterial strains that were researched for SBSLS control could be applied to depress this disease and reveal more resistant plants as well (Mrabet *et al*., 2011; Slimene *et al*., 2012). Jasinski *et al*. (2009) showed that *Medicago* spp. genotypes different by resistance also differs by concentration of antimicrobial compounds. This relation could be employed for selection of more resistant accessions.

Resistance reaction was much clear in 2009 when alfalfa nursery was established in the middle of summer and period of disease development was much shorter compared to 2010 and 2011. The main problem for more resistant genotypes selection is too high disease pressure. Therefore, establishing of nurseries in middle of summer in every year also could support resistance breeding. The second part of summer should be selected avoiding dry weather of May and June as August and September are characterized by excessive precipitations and very abundant dew. During couple month of vegetation resistant plants could be infected enough to select them among the rest plants at the same time they should not be infected too much as happened in 2010 and 2011.

Seed will not mature at the same year and selected plants should be evaluated for the further seasons to evaluate resistance to Sclerotinia crown and stem rot (*Sclerotinia trifoliorum*) in spring as well as to downy mildew during the same and further seasons. As only alfalfa cultivars possessing complex resistance to a range of pathogen can be successfully grown in wet and cool climate of the Baltic Sea region countries.

Conclusions

Tested material did not possess suitable level resistance to SBSLS. Selection of material promising by resistance should be done under conditions moderately favourable to development of this disease. Conversely, propagation of selected genotypes is hardly possible without additional protection measures.

References

Akamatsu, H.O., M.I. Chilvers and T.L. Peever. 2008. First report of spring black stem and leaf spot in Washington State caused by *Phoma medicaginis*. *Plant Dis*., 92: 833.

Anonymous. 2012a. II. Fodder plants. *Medicago sativa* L. In: *Common Catalogue of Varieties of Agricultural Plant Species 31*, pp. 132-140.

Anonymous. 2012b. Lithuanian statistical department. http://db1.stat.gov.lt.

Barbetti, M.J. 2007. Resistance in annual *Medicago* spp. to *Phoma medicaginis* and *Leptosphaerulina trifolii* and its relationship to induced production of a phytoestrogen. *Plant Dis*., 91: 239-244.

Campbell, C.L. and L.V. Madden. 1990. *Introduction to plant disease epidemiology*. John Wiley & Sons, New York, USA.

Castell-Miller, C.V., L.J. Szabo, L.R. Gale, N.R. O'Neil and D.A. Samac. 2008. Molecular variability of Minnesota population of *Phoma medicaginis* var. *medicaginis*, the causal agent of spring black stem and leaf spot of alfalfa. *Can. J. Plant Pathol*., 30: 85-96.

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

Djebali, N. 2013. Aggressiveness and host range of *Phoma medicaginis* isolated from *Medicago* species growing in Tunisia. *Phytopathol. Mediterr*., 52: 3-15.

Ellwood, S.R., L.G. Kamphuis and R.P. Oliver. 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.

Hanson, A.A. 1998. Alfalfa and alfalfa improvement. American Society of Agronomy, Madison, USA.

Jasinski, M., P. Kachlicki, P. Rodziewicz, M. Figlerowicz and M. Stobiecki. 2009. Changes in the profile of flavonoid accumulation in *Medicago truncatula* leaves during infection with fungal pathogen *Phoma medicaginis*. *Plant Physiol. Bioch*., 47: 847-853.

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

Kamphuis, L.G., J. Lichtenzveig, R.P. Oliver and E.S.R. Ellwood. 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., Y. Mizukami and F. Fujimoto. 2002. Improvement of resistance to Sclerotinia crown and stem rot of alfalfa through phenotypic recurrent selection. *Jpn. Agr. Res*., pp. 36: 1-5.

Lamb, J.F.S., C.C. Sheaffer, L.H. Rhodes, R.M. Sulc, D.J. Undersander and E.C. Brummer. 2006. Five decades of alfalfa cultivars improvement: impact on forage yield, persistence, and nutritive value. *Crop Sci*., 46: 902-906.

Liatukienė, A. 2012. *Investigation of genetic diversity of lucerne (Medicago spp.) by identifying resistance to pathogen and mobile aluminium*. Ph. D Thesis, Akademija, Lithuania.

Mosimnan, E. and J. Lehmann. 2002. Management of grass/legumes mixtures for a three year duration. *Grassland Sci. Eur*., 7: 454-455.

Mrabet, M., E. Abdellatif, K. Zribi, R. Mhamdi and N. Djebali.

2011: *Sinorhizobium meliloti* can protect *Medicago truncatula* from infection by *Phoma medicaginis*. *Phytopathol. Mediterr*., 50: 183-191.

Nekrošius, A., K. Navickas, K. Venslauskas, Ž. Kadžiulienė and V. Tilvikienė. 2014. Assessment of energy biomass potential and greenhouse gas emissions from biogas production from perennial grasses. *Agriculture-Zemdirbyste*, 101: 271-278.

Nutter, F.R., J. Guan, A.R. Gotlieb, L.H. Rhodes, C.R. Grau and R.M. Sulc. 2002. Quantifying alfalfa yield losses caused by foliar diseases in Iowa, Ohio, Wisconsin, and Vermont. *Plant Dis*., 86: 269-277.

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

Rashidi, M., B. Zand and M. Gholami. 2009. Effect of different seeding rates on seed yield and some seed yield components of alfalfa (*Medicago sativa*). *Int. J. Agric. Biol*., 11: 779-782.

Rodriguez, R., K.T. Leath and R.R. Hill. 1990. Pathogenicity of *Phoma medicaginis* var. *medicaginis* to root of alfalfa. *Plant Dis*., 74: 680-683.

Šlepetys, J. 2008. Productivity and persistence of pure and mixed forage swards. *Latv. J. Agric*., 11: 276-282.

Slimene, I.B., O. Tabbene, N. Djebali, P. Cosette, J.M. Schmitter, T. Jouenne, M.C. Urdaci and F. Limam. 2012. Putative use of *Bacillus subtilis* L194 strain for biocontrol of *Phoma medicaginis* in Medicago truncatula seedlings. *Res. Microbiol*., 163: 388-397.

Wang, H., S.F. Hwang, K.F. Chang, B.D. Gossen, G.D. Turnbull and R.J. Howard. 2004. Assessing resistance to spring black stem and leaf spot of alfalfa caused by *Phoma* spp. *Can. J. Plant Sci*., 84: 311-317.

Wunsch, M.J., K.A. Bassendowski, G.C. Bergstrom and B.D. Gossen. 2010. In: *Incidence of foliar infection of alfalfa by Phoma medicaginis and P. sclerotioides in Saskatchewan, New York, and Vermont in 2008 and 2009*. (Ed.): A. O'Shea. Canadian Plant Disease Survey, Hogg Crescent, Saskatchewan, Canada, pp. 116-118.

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