INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–575/2014/16–5–905–910
http://www.fspublishers.org

Full Length Article

Downy Mildew Reaction of Alfalfa Accessions of Different Geographical Origin under Lithuanian Conditions

Aurelija Liatukienė and Žilvinas Liatukas*
Institute of Agriculture, Research Centre for Agriculture and Forestry, Instituto av. 1, Akademija, Lithuania
*For correspondence: liatukas@lzi.lt

Abstract

Study was conducted during 2009–2011 under field conditions with natural infection at the Institute of Agriculture located in central part of Lithuania. The alfalfa (*Medicago sativa* L.) downy mildew (caused by *Peronospora trifoliorum*) resistance was evaluated on 100 accessions originating from distinct countries across the world. Wet weather conditions were highly favourable for alfalfa downy mildew resistance investigations. Accessions were compared by maximal disease severity (DS) and area under disease progress curve (AUDPC). Disease development was very intensive in 2009 when DS ranged from 8–80% and AUDPC value ranged 140–1938. DS but not AUDPC values was lower in 2010, DS ranged from 6.3–45% and AUDPC ranged 203-2123. The lowest disease development determined in 2011 when DS ranged 1.8–17.5% and AUDPC ranged 40–266. The relatively stable disease development on accessions possessing different resistance was indicated by medium to very strong (r=0.587* – 0.932**) correlation coefficients between DS and AUDPC across years. Origin of the host accessions showed considerable impact on resistance. According to data of 2009, resistant and medium resistant (DS up to 10 and 20%, respectively) accessions accounted for 7 and 13%, respectively. The majority of the most resistance accessions originated from neighbouring countries characterized by similar to Lithuanian cool temperate climate. © 2014 Friends Science Publishers

Keywords: *Medicago sativa*; Resistance; *Peronospora 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 species presents large diversity for various traits since it is cultivated in contrasting environments (Julier *et al*., 2000). The recent trend of increasing prices for fertilizers, especially nitrogen, 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 legume grasses in Lithuania also (Šlepetys, 2008), but growing area compose small share among total area of grasses (Anonymous, 2012b). The recent investigation of alfalfa disease resistance in Lithuania showed that broad range of diseases can heavily damage all plant parts of alfalfa in Lithuania (Liatukienė and Liatukas, 2010). The highest negative impact diseases make on 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 (Anonymous, 2012a). Whereas under dry hot climate conditions alfalfa produces high seed yields (Rashidi *et al.,* 2009).

Downy mildew, caused by *Peronospora trifoliorum* deBy., is harmful disease of alfalfa in the temperate climate areas. The most efficient mean to control disease is growing of resistant cultivars. Investigations of alfalfa resistance to fungal diseases showed that material of different origin were considerably different by resistance to downy mildew (Jie *et al*., 2000; Yaege and Stuteville, 2000). Genetic peculiarities of resistance to *P. trifoliorum* were comprehensively investigated in studies of Skinner and Stuteville (1985; 1988). Availability of genetically diverse alfalfa material allows developing of alfalfa cultivars improved by disease resistance (Nagl *et al*., 2011).

Study of Lamb *et al.* (2006) showed that cultivars yielding improvement during 50 years of breeding 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.

Information about alfalfa cultivars resistance to downy mildew in Europe is scanty, only some indirect studies are available. Comprehensive recent research including considerable number of accessions was not found. Therefore, the present study aimed to determine the downy mildew resistance of geographically different alfalfa accessions under cool temperate climate conditions of Lithuania.

To cite this paper: Liatukiene, A. and Ž. Liatukas, 2014. Downy mildew reaction of alfalfa accessions of different geographical origin under Lithuanian conditions. *Int. J. Agric. Biol.,* 16: 905–910

Materials 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 CambisolC Mg-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 nursery was established 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 geographical origin (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

Downy mildew was evaluated in 2009-2011. Disease severity (DS) was evaluated during all season by using the scale: 0, 0.1, 1, 5, 10, 20, 40, 60, and 80% (Campbell and Madden, 1990). The resistance level of accessions was compared by maximal DS in 2009 that varied from 8.0 to 80.0%. Accessions evaluated by DS up to 10.0% were considered asresistant (R), >10.0–20.0% as medium resistant (MR), >20.0–40.0% as medium susceptible (MS), >40.0–60.0% as susceptible(S), and over 60.0% as highly susceptible (HS).

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. January was very cold with weak snow cover in 2010. Nonetheless, alfalfa over wintering 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.

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 and Madden, 1990).
Statistical calculations were done using ANOVA.

Results

Development of Downy Mildew

The downy mildew was the first disease that started after renewal vegetation of alfalfa. However, its development later was stopped by other diseases in 2010–2011, especially by spring black stem and leaf spots (causal agent *Phoma medicaginis* var. *medicaginis*) (Table 2). Fig. 1 shows the downy mildew development on three alfalfa genotypes considerably differing in AUDPC values in 2009-2011. The downy mildew severity constantly decreased from 2009 to 2011. AUDPC values among the most resistant and susceptible alfalfa genotype differed about 10-fold from 159 to 1820 and 218 to 2123 in 2009–2010, respectively. The difference among AUDPC values was about 5-fold from 49 to 219 in 2011. Maximal disease severity among alfalfa genotypes differed similarly. Severe downy mildew development on susceptible cultivars in 2009 during couple of months after sowing 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 severity in resistant cultivar (8.0, 6.8 and 1.8% in 2009–2011, respectively) shows sound impact of the resistant cultivars use in the control of downy mildew.

Alfalfa Downy Mildew Reaction

The accessions presented in Table 3 are sorted in ascending order of maximal downy mildew severity in 2009. The alfalfa accessions differed considerably in downy mildew severities. The screening revealed similar differentiation of alfalfa resistance when DS and AUDPC values were compared in all years. The both values can be used to estimate alfalfa resistance. However, calculation of AUDPC values can be done only after several DS assessments. On the other hand, downy mildew development (Fig. 1) in 2009–2011 showed necessity to evaluate disease development as longer as possible. Maximal DS was registered in different disease development periods. This value was registered at the last assessment in 2009 and 2011 and at the second assessment in 2010.

Table 1: Precipitations and temperature in 2009–2011, (Lithuania, Akademija weather station)

Month	Precipitations, mm				Temperature (℃)			
	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

Table 2: Diseases of alfalfa in 2009–2011

Disease	Year		
	2009	2010	2011
Downy mildew	+++	++	++
Spring black stem and leaf spot	+	+++	+++
Sclerotinia crown and root rot	-	+	++

-no disease, +low severity, ++ medium severity, +++ high severity

The maximal DS ranged from 8.0 to 80.0, 6.3 to 35.0 and 1.8 to 17.5% in 2009–2011, respectively. The AUDPC values ranged from 140 to 1838, 203 to 2123 and 40 to 266 in 2009-2011, respectively.

Origin of the host accessions showed considerable impact on resistance. Alfalfa accessions are compared by results of 2009, due to the highest differentiation of alfalfa accessions downy mildew resistance in 2009. Köppen-Geiger climate zone classification (Peel *et al.*, 2007) was only partially applicable for grouping of alfalfa accessions by downy mildew resistance. The resistant (7%) and medium resistant (18%) accessions originated from Lithuania and neighbouring countries with wet and cool weather. Only cultivars Romagnola (R) and Picweeh (MR) originated from geographically distinct countries (Italy and Canada, respectively) characterized by rather dry weather during plant vegetation. The medium susceptible accessions (13%) group contained cultivars originated from countries which can be characterized as similar to above mentioned. The sound difference regarding geographical origin was clear in the group of susceptible accessions (30%). Only about a third of accessions originated from countries with rather cool and wet climate. The rest two thirds originated from countries characterized by rather or clearly dry and warm or hot weather. The highly susceptible accessions (32%) originated from countries characterized by rather or clearly dry and warm or hot weather. Some exception can be accessions PI 214218 (Denmark), PI 577507 and PI 502485 (Germany). However, the main reason for this exception should be old development data of these accessions.

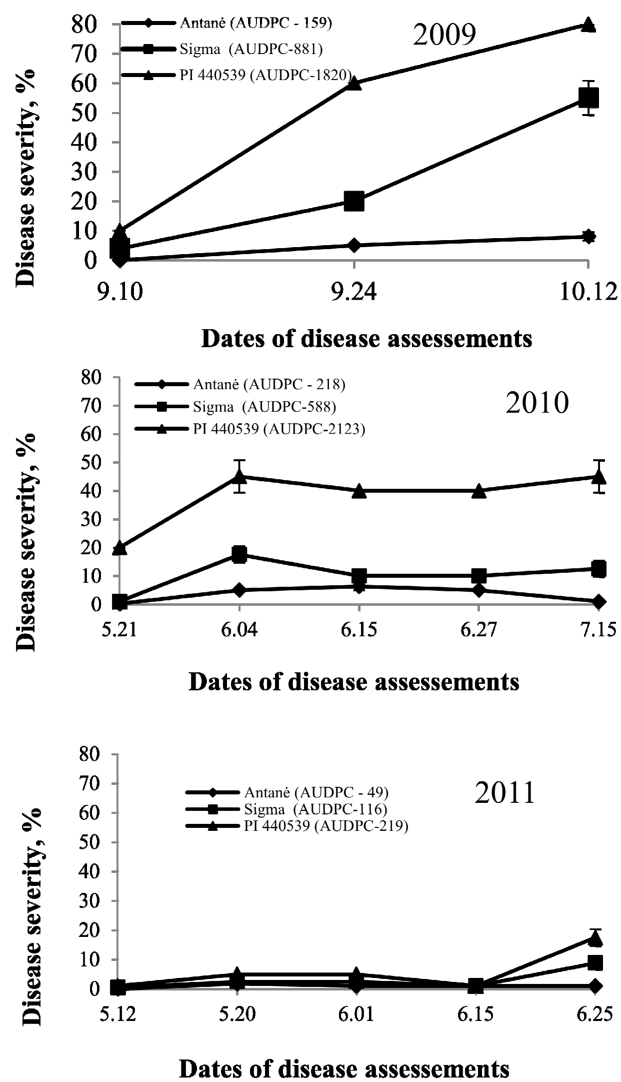

Fig. 1: Development of downy mildew in alfalfa cultivars with differentAUDPC values in 2009–2011

The correlations between DS and AUDPC results of accessions across years varied from medium to very strong (r=0.587*– 0.932**) (Table 4). The correlations between DS and AUDPC of the same year were strong to very strong (r=0.838** –0.932**).The correlations between DS of different year as well as AUDPC were medium to strong (r=0.654*–0.741** and 0.587*–0.816**, respectively). The same tendency was calculated for correlations between DS and AUDPC of different years (r=0.625*–0.822**).

Discussion

Development and severity of downy mildew and cultivars differentiation shows that Lithuanian climate is very favourable for alfalfa resistance investigations.

Table 3: Maximal severities and AUDPC values of downy mildew in alfalfa accessions in 2009–2011

Alfalfa accession	Country of origin	Maximal disease severity, %			AUDPC value		
		2009	2010	2011	2009	2010	2011
Antanė	LT*	8.0 a**	6.3a	1.8ab	159ab	218ab	48ab
Radius	PL	8.0a	10.0ab	2.8b	159ab	279a-c	62bc
Nadezhda II	RU	10.0ab	10.0ab	1.8ab	140a	279a-c	62bc
Žydrūnė	LT	10.0ab	6.3a	1.8ab	178ab	218ab	50b
Birutė	LT	10.0ab	6.3a	1.8ab	178ab	203a	58b
Vertus	SE	10.0ab	8.8ab	5.0c	194a-c	252a-c	106de
Romagnola	IT	10.0ab	6.3a	1.8ab	230bc	240ab	48ab
Bayard	FR	12.0a-c	8.8ab	1.8ab	159ab	247ab	50b
Malvina	LT	12.0a-c	6.3a	1.8ab	197a-c	203a	50b
Karlu	EE	12.0a-c	6.3a	1.8ab	197a-c	224ab	44ab
Saartepola	EE	12.0a-c	10.0ab	6.3c	197a-c	365cd	67bc
Augūnė	LT	12.0a-c	8.8ab	1.0a	253bc	246ab	40a
Vorksla	RU	12.0a-c	8.8ab	1.8ab	285bc	262a-c	52b
Bella	NL	12.0a-c	17.5cd	5.0c	286bc	414de	122fg
Resis	DK	18.0b-d	10.0ab	2.8b	271bc	388c-e	67bc
Lucia	SK	18.0b-d	10.0ab	2.8b	305bc	279a-c	77b-d
Janu	NL	18.0b-d	12.5bc	5.0c	314b-d	382c-e	143g-i
Jurlu	EE	20.0cd	10.0ab	1.8ab	273bc	393c-e	44ab
Tagamorsa	EE	20.0cd	10.0ab	6.3c	273bc	376c-e	67bc
Luna	BE	20.0 cd	8.8ab	2.8b	290bc	322b-d	61b
Magda	CZ	20.0cd	17.5cd	5.0c	324b-d	416de	118fg
Bagira	RU	20.0cd	8.8ab	2.8b	326b-d	248ab	67bc
Jogeva 118	EE	20.0cd	10.0ab	1.8ab	326b-d	352cd	44ab
Vela	DK	20.0cd	8.8ab	1.8ab	362b-d	263a-c	62bc
Picweeh	CA	20.0cd	8.8ab	1.8ab	362b-d	288bc	52b
Mazhotnes	BY	25.0de	10.0ab	2.8b	337b-d	388c-e	67bc
Niva	CZ	25.0de	10.0ab	2.8b	344b-d	352cd	94d
Orca	FR	25.0de	17.5cd	5.0c	371cd	421de	71b-d
Kunsmme	EE	25.0de	10.0ab	1.8ab	377cd	283a-c	44ab
Vilsana	EE	25.0de	12.5bc	5.0 c	377cd	325b-d	60b
Jarka	CZ	25.0de	12.5bc	2.8b	410c-e	340cd	73b-d
Viktorija	CZ	35.0e-g	10.0ab	5.0c	439c-e	302b-d	106de
Morova	CZ	35.0e-g	8.8ab	1.8ab	466de	288a-c	63bc
Ellerskie I	CA	35.0e-g	6.3a	1.8ab	468de	240ab	48ab
EerikSaare	EE	35.0e-g	10.0ab	6.3c	470de	310b-d	67bc
Magali	FR	35.0e-g	12.5bc	2.8b	472de	466ef	77b-d
Palava	CZ	35.0e-g	10.0ab	5.0c	482d-f	312b-d	108ef
Semira	RU	35.0e-g	35.0f	8.8d	505d-f	1084ij	143g-i
Marija Odd.	UA	45.0fg	8.8ab	2.8b	527ef	295bc	63bc
Luzelle	FR	45.0fg	17.5cd	2.8b	527ef	445d-f	68bc
Abruka	EE	45.0fg	12.5bc	6.3c	528ef	334cd	76b-d
Verko	HU	45.0fg	10.0ab	1.0a	534ef	319b-d	40a
Mireille	FR	45.0fg	25.0e	5.0c	563e-g	567f-h	193i-k
Algongnin	CA	45.0fg	17.5cd	2.8b	567e-g	459d-f	67bc
Commandor	USA	45.0 fg	10.0ab	2.8b	600e-g	336cd	77b-d
PI 206283	TR	45.0fg	35.0f	6.3c	600e-g	833hi	162hi
Zuzana	CZ	45.0fg	8.8ab	2.8b	621fg	297bc	84 cd
PI 422567	RU	45.0fg	20.0d	12.5e	626 fg	833hi	189ij
Bobrava	CZ	45.0fg	17.5cd	2.8b	793hi	416de	111ef
Creno	DK	45.0fg	35.0f	2.8b	793 hi	681gh	72b-d
Kardla	EE	55.0hi	10.0ab	2.8b	655f-h	354cd	49b
Jitka	CZ	55.0hi	17.5cd	2.8b	658 f-h	416de	77b-d
Sitel	NL	55.0hi	17.5cd	1.8ab	722gh	414de	45ab
Alfagraze	CA	55.0hi	10.0ab	2.8 b	726gh	331cd	73b-d
Europe	FR	55.0hi	12.5bc	2.8b	860ij	356cd	67bc
Sigma	RO	55.0hi	17.5cd	8.8d	881ij	588f-h	116fg
PI 211609	AF	55.0hi	35.0f	8.8d	1211lm	1867m	145g-i
PI 467888	USA	55.0hi	35.0f	8.8d	1358l-n	1204jk	108ef
Plauresa	DE	60.0i	12.5bc	6.3c	709gh	538fg	85cd
Belfeuil	FR	60.0i	17.5cd	2.8b	742g-i	435d-f	88cd
Sandra	RO	60.0i	17.5cd	6.3c	742g-i	588f-h	108ef
Magnat	RO	60.0i	17.5cd	6.3c	775hi	490e-g	95d
Vertibenda	PL	60.0i	25.0e	8.8d	874ij	836hi	130fg
Polder	FR	60.0i	20.0d	2.8b	885ij	526fg	90cd
Derby	NL	60.0i	20.0d	2.8b	885ij	465ef	68bc
Mandolina	RO	60.0i	20.0d	8.8d	885ij	563f-h	98de
PI 573153	CN	60.0i	35.0f	6.3c	1025j-l	968h-j	172h-j
Elda	EE	60.0i	12.5bc	6.3c	1155k-m	465ef	67bc
Daniela	RO	65.0ij	20.0d	6.3c	717gh	520e-g	107de
Kosmina	RO	65.0ij	20.0d	6.3c	750g-i	526fg	107ef
Natsuwakaba	JP	65.0ij	17.5cd	8.8d	75g-i	66gh	142gh
Luxin	RO	65.0ij	20.0d	5.0c	790hi	603f-h	124fg
PI 499547	CN	65.0ij	35.0f	10.0d	1037j-l	1135i-k	204i-k
Alina	RO	65.0ij	25.0e	6.3c	103 j-l	765g-i	101de
PI 467980	USA	65.0ij	35.0f	8.8d	1230lm	1134i-k	110ef
Pulav	UA	75.0j-l	12.5bc	8.8d	796hi	563f-h	172h-j
Tin Jin	CN	75.0 j-l	35.0f	8.8d	918i-k	1304j-l	132gh
Szarvasi	HU	75.0 j-l	25.0e	8.8d	1017j-l	769g-i	120fg
Mediterranea	ES	75.0 j-l	35.0f	6.6c	1060j-l	1012h-j	83cd
PI 452463	CA	75.0j-l	35.0f	10.0d	1136k-m	1072ij	178ij
PI 214218	DK	75.0j-l	35.0f	8.8d	1160k-m	975h-j	143g-i
PI 577507	GE	75.0j-l	20.0d	6.3c	1160k-m	848hi	143g-i
PI 452444	USA	75.0j-l	25.0e	12.5e	1308k-m	1084ij	139gh
PI 502485	GE	75.0j-l	35.0f	6.3c	1318k-m	988h-j	118fg
PI 467916	USA	75.0j-l	35.0f	8.8d	1655mn	1195 i-k	123fg
PI 467899	USA	75.0j-l	35.0f	6.3c	1755no	1315 j-l	95d
Adin	RO	80.0kl	17.5cd	5.0c	860ij	568f-h	97de
PI 577514	RU	80.0kl	25.0e	6.3c	899i-k	945h-j	162hi
PI 467895	USA	80.0kl	25.0e	6.3c	932i-k	1162i-k	108ef
Katinka	RO	80.0kl	25.0e	5.0c	965jk	663gh	154hi
PI 212104	AF	80.0kl	35.0f	10.0d	1094kl	1438 kl	191i-k
PI 573153	CN	80.0kl	35.0f	17.5g	1097kl	1523k-m	266jk
PI 577460	PK	80.0kl	45.0h	8.8d	1160k-m	1589lm	175h-j
PI 467910	USA	80.0kl	25.0e	8.8d	1180k-m	786g-i	127fg
PI 467901	USA	80.0kl	25.0e	10.0d	1180k-m	786g-i	126fg
Rancap	PE	80.0kl	35.0f	5.0c	1573mn	1004h-j	87cd
PI 467965	USA	80.0kl	35.0f	6.3c	1703no	1207i-k	137gh
PI 440539	KZ	80.0kl	45.0h	17.5g	1820n-p	2123n	219i-k
PI 467922	USA	80.0kl	35.0f	10.0d	1820n-p	1066ij	133gh
PI 449316	CN	80.0kl	35.0f	12.5e	1838n-p	1165i-k	178ij
Average		47.4	19.4	5.4	724.8	621.8	100.6

*AF – Afghanistan(2), BE – Belgium(1), BY – Belarus(1), CA – Canada(5), CN – China(5), CZ – Czech Republic(9), DE – Germany(1), DK – Denmark(4), EE – Estonia(11), ES – Spain (1), FR – France(8) GE – Georgia(2), HU – Hungary(2), IT – Italy(1), JP – Japanese(1), KZ – Kazakhstan(1), LT – Lithuania(5), NL – Netherlands(4), PE – Peru(1), PK – Pakistan (1),PL – Poland(2), RO – Romania(10), SE – Sweden(1), SK – Slovakia(1), RU – Russia(6), TR – Turkey(1), UA – Ukraine(2), USA – Unites States of America (11). Accessions number per country is shown in brackets

**Means fallowed by the same letters do not differ according to Duncan's Multiple Range Test at probability P<0.01

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.

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 (Hanson, 1998). Usually it damages top of plants. When infection is severe, very susceptible plants can be destroyed completely just after regrowth in spring.

Table 4: Correlation among downy mildew disease severities (DS) and AUDPC values in 2009–2011

Traits	2009-DS	2010-DS	2011-DS	2009-AUDPC	2010-AUDPC
2010–DS	0.741**				
2011–DS	0.654**	0.731**			
2009–AUDPC	0.885**	0.822**	0.685*		
2010–AUDPC	0.723**	0.932**	0.805**	0.816**	
2011–AUDPC	0.624*	0.735**	0.838**	0.587*	0.755**

*p<0.05, **<0.01

Top damage of plants made highly negative impact to seed production even under relatively low disease severity in 2010 and 2011, in all susceptible 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 (Skinner and Stuteville, 1988; 1989; Skinner and Stuteville, 1992; Yaege and Stuteville, 2000). Since alfalfa is cross pollinating plant, it populations consists of plants which vary by resistance (Skinner and Stuteville, 1985; 1989). In our case, we did not find any accessions consisting of completely resistant individuals as well as in the study of Skinner and Stuteville (1992) with several hundreds of *Medicago* spp. accessions. However, the most resistant accessions were damaged only up to 8.0% in 2009. It shows possibility to select individual plants with highest resistance across population and develop new improved populations. Development of new populations with considerably higher resistance level takes several selection cycles which can continue up to 10 and more years (Kanbe *et al*., 2002). Screening populations resistant at seedling stage can denote the most resistant seedlings (Yaege and Stuteville, 2000). However, disease agent can adapt to monogenes very rapidly. Therefore, a more promising resistance breeding strategy should rely on accumulation of polygenes (Slusarenko *et al*., 2000). It means that selection should be relying on multiple assessment data received under field conditions. Greenhouse growing technology can be applied but it requires very high inputs. Lithuanian weather conditions are very favourable for *P. trifoliorum* spread and development as wet years are most common. Alfalfa nurseries should be established in the second part of summer avoiding dry weather of May and June as August and September are characterized by excessive precipitations and very abundant dew. Plants vegetation period during August and September is long enough for considerable disease development and further selection of resistant plants in populations. Seed will not mature at the same year but in any way, selected plants should be evaluated for the 2nd season to evaluate resistance to *Sclerotinia* crown and stem rot (*Sclerotinia trifoliorum*) in spring as well as to spring black stem and leaf spots during summer. 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 countries.

Previous 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). Our experimental data was in accordance with these studies. It means that geographically distinct accessions could hardly improve downy mildew resistance of Lithuanian or neighbouring countries alfalfa breeding material. It is serious constraint for introduction of useful traits such as tolerance to aluminium. Intensive alfalfa aluminium tolerance breeding is progressing across a range of countries (Scott *et al*., 2008; Vitorello *et al*., 2005). Very promising material is developed but in most cases, it does not possess resistance to complex of diseases that are harmful in Baltic Sea region (Liatukienė, 2012).

In conclusion, selection of the most diseases resistant plants from such material and later crossing with locally adapted material should enable introductions of such traits. The majority of Baltic Sea region alfalfa material was resistant or medium resistant to downy mildew. These alfalfa accessions can be used to improve downy mildew resistance in the other countries.

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(Received 29 April 2013; Accepted 23 December 2013)