

Reaction of lucerne (*Medicago* spp.) to fungal diseases caused by *Phoma medicaginis*

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Thirty-seven accessions (cultivars and populations) of *M. sativa*, *M. varia* and *M. falcata* (*Medicago* spp.) in a vegetative pot experiment and 46 accessions of *Medicago* spp. in a field experiment under natural infection were evaluated for *Phoma medicaginis* resistance at the Lithuanian Institute of Agriculture during 2008–2009. The results showed that the accessions possessed a broad range of partial resistance. The most resistant accessions had spring black stem and leaf spot (SBSLS) areas under the disease progress curve (AUDPC) values ranging within 429–868, whereas the most susceptible ones showed the AUDPC values of 2060–2650. All four disease severity evaluation values strongly correlated ($r = 0.806^{**}$ – 0.968^{**}) (*, ** $P < 0.05, 0.01$) with the AUDPC values. Disease severity in 2008 strongly correlated ($r = 0.906^{**}$) with AUDPC in 2009 under field conditions, whereas SBSLS AUDPC correlated weaker ($r = 0.589^{**}$) with DS under pot conditions in 2008. Susceptibility to SBSLS was negatively strong and medium-correlating with pod setting ($r = -0.716^{**}$), overwintering ($r = -0.584^*$), crop rankness ($r = -0.544^*$), grass yield ($r = -0.576^*$) and stem thickness ($r = -0.530^*$).

Key words: *Medicago*, *Phoma medicaginis*, resistance, AUDPC

INTRODUCTION

Lucerne (*Medicago* spp.) is an important forage crop grown worldwide. Spring black stem and leaf spot (SBSLS), caused by *Phoma medicaginis* var. *medicaginis* Malbr. & Roum., is one of the most harmful fungal diseases in temperate regions of North America and Europe (Castell-Miller et al., 2007; Leyronas et al., 2004; Wang et al., 2004). This disease damages all plant parts: roots, crowns, stems, leaves, flowers, pods and seeds. The highest negative effects are determined for above-ground plant parts (Fonseca et al., 1999; Hwang et al., 2006; Leyronas et al., 2004). The loss of forage yield fluctuates from several to as high as 50%, depending on location and year (Campbell, Duthie, 1990; Nutter et al., 2002). The forage quality traits are affected negatively, too (Barbetti, 2007; Fonseca et al., 1999; Guan, Nutter, 2002). Seed yield and its quality also suffer a decrease and in some cases can be lost totally if very susceptible cultivars are cultivated (Barbetti, 1995; Barbetti, Nichols, 1991). This disease also causes crown and root rot. It decreases crop stand density and profitable crop use age (Rodriguez, 2005; Rodriguez, Leath, 1992). Forage losses partially can be avoided by early cutting, but this praxis is not available in the case of seed production. The most efficient control of this disease is cultivation of resistant cultivars because chemical and cultural control has a low efficiency (Campbell, Duthie, 1990; Duthie, Campbell, 1991; Hwang et al., 2006; Nutter et al., 2002). However, the

frequency of *P. medicaginis* resistant populations as well as of resistant individuals within populations is low, and highly resistant cultivars are not available commercially. Meanwhile, some resistant germplasm is available among landraces, related species and other exotic sources (Castell-Miller et al., 2007; Ellwood et al., 2006; Kamphuis et al., 2008; Wang et al., 2004).

Resistance breeding is complicated not only by the deficiency of highly resistance germplasm, but also due to quantitative inheritance of resistance. This type of resistance in combination with cross-pollination of lucerne requires a long time and resources to develop new more resistant populations (Djebali et al., 2007; Ellwood et al., 2006; Kamphuis et al., 2008; O'Neil et al., 2003). Some facilitation is allowed by the low genetical variability of *P. medicaginis* isolates from different geographical localities and separate plant organs (Castell-Miller et al., 2008). The pathogenicity and virulence of the majority of isolates do not differ significantly, but selection of isolates by the level of aggressiveness and virulence is advisable (Gray et al., 1990; Rodriguez, 2005). Medium to high correlations of resistance reactions during different plant development stages allow to test breeding material under laboratory, greenhouse or field conditions and accelerate the development of resistant cultivars (O'Neil et al., 2003; Wang et al., 2004).

The objective of this study was to determine the reaction of lucerne accessions to *P. medicaginis*.

MATERIALS AND METHODS

The experiment was carried out at the Lithuanian Institute of Agriculture (LIA) in 2008–2009 under field and vegetative pot conditions. The experimental material consisted of 37 accessions (cultivars and populations) of *M. sativa*, *M. varia* and *M. falcata* (*Medicago* spp.) for the vegetative pot experiment and 46 accessions of *Medicago* spp. for the field experiment under natural infection.

Accessions for the vegetative pot experiment were seeded in April 2008 with scarified, *Rhizobium* not-inoculated 30 seeds separately in plastic pots filled with 10 l of a mix of soil and peat moss substrate with pH 7.0 (1 : 1). Each accession was seeded into three pots and arranged together in a greenhouse. Pests were controlled when appeared. Ventilation was adjusted to avoid air temperatures above 28 °C. Pots were watered to field moisture capacity on a daily basis. The seedlings were thinned at the end of the 3rd week; ten most vigorous seedlings in each pot were left for further evaluations. Plants were fertilized with a complex of nutrient elements. The mixture consisted of nitrogen, phosphorus, potassium, sulphur and magnesium (1.0 : 0.5 : 1.0 : 0.5 : 0.25). Fertilisation was done three times at post-germination, mid-vegetative and early flower stages at a rate of 3 g per pot. After seed ripening, the plants were cut down and brought outside of the greenhouse. The pots were brought back to the greenhouse after regrowth when the first symptoms of SBSLS became visible on upper leaves. Evaluation of SBSLS severity was done after development of severe disease symptoms on susceptible accessions.

The lucerne nursery for the field experiment was sown after black fallow without a cover crop in July 2008. The soil of the experimental site is *Endocalcari-Endohypogleyic Cambisol* CMg-n-w-can (pH 7.2–7.5, P₂O₅ 201–270 mg kg⁻¹ and K₂O 101–175 mg kg⁻¹, humus 2.46%). P₆₀K₉₀ was applied pre-sowing. The plots were sprayed with herbicides (Basagran 480, 2 l ha⁻¹) when lucerne after germination reached the height of 10 cm. Insecticides (Karate Zeon 5 CS, 0.15 l ha⁻¹) were applied when pests became harmful. Every breeding population was sown in two 5-metre long rows. The sowing rate was 50 scarified seeds per 1 metre. The distance between the rows of a line was 0.5 m; the distance between different lines was 1.0 m. The seedlings were thinned at the end of the 3rd week; ten most vigorous seedlings per metre were left for further evaluations. Every accession was evaluated for all traits as one unit. Lucerne accessions were evaluated for SBSLS resistance in the autumn of the establishment year.

The agro-morphological features were evaluated during the vegetation season in 2009. Overwintering was evaluated after vegetation resumption. Resistance to *Sclerotinia* stem and crown rot (SSCR) was evaluated two weeks after vegetation resumption. Crop equality and rankness, the amount of leaves and grass yield, stem density, thickness, colour, growth habit were evaluated at the beginning of flowering

on a 5-point scale, 1 being the lowest value. Plant height was measured in centimetres two weeks after vegetation resumption (spring regrowth), at the bud stage, and during full flowering. Flower colour, the amount and shape of inflorescences was measured during full flowering on the same scale. The length and width of the terminal leaflet were measured in millimetres. A SPAD-502 leaf chlorophyll (Chl) meter was used to obtain a measure of Chl concentration of 20 upper well-developed leaves per accession; data are presented as values of this device. Chlorophyll concentration was measured three times: at the bud stage, at the beginning of flowering, and during full flowering. Dry matter % was evaluated in the 1st and 2nd grass yields. The 1st grass was cut at the beginning of flowering and the 2nd at the bud stage. Nitrogen % was evaluated in dry matter of the 1st grass of 7 most promising accessions by the Kjeldahl method.

SBSLS severity was evaluated at the beginning, full middle and end of flowering in % using the following 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.

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

Data were analyzed using Excel software. The correlation-regression analysis was used to evaluate the relationships among the traits. The obtained correlation coefficients were compared for significance level at $P < 0.05$ and $P < 0.01$.

The sowing year was favourable for the establishment of plants. The wet and long autumn was favourable for an intensive spread and development of SBSLS. The winter of 2006 was mild, and the winterhardness of the genotypes mostly depended on their resistance to root, crown and stem rots. The spring was dryer than usual. However, frequent and abundant precipitations during June and July in 2009 were very favourable for an intensive spread and development of SBSLS.

RESULTS AND DISCUSSION

Figure 1 shows the SBSLS severity on lucerne accessions with contrasting resistance. The most resistant accession LIA3023 during the first evaluation had SBSLS severity of only 0.1%, whereas the most susceptible LIA3030 was damaged up to 7.5%. Accessions during the 4th evaluation were damaged from 40 (LIA2234) to 80% (LIA3037).

The relationship between disease severity (DS) and AUDPC is shown in Fig. 2. A correlation between DS and AUDPC was strong ($r = 0.806^{**}-0.968^{**}$) for all diseases. The variability of AUDPC was high for accessions with the same DS at all disease evaluations. The variability of the AUDPC value was higher for more resistant accessions than that for more susceptible ones. At the 1st disease evaluation,

accessions with DS 0.1% had AUDPC 791–1246, whereas those with DS 10.0% 1757–2565. The variability AUDPC at the 2nd DS evaluation was somewhat lower as accessions with DS 5% had AUDPC 429–1212 and accessions with DS 30% had AUDPC 1918–2395. Similar relations were found while comparing AUDPC with DS at the 3rd and

the 4th evaluations. Data presented in Fig. 2 also show the distribution curve which is close to a normal distribution of accessions in SBSLS resistance. This distribution is typical of populations possessing a partial resistance type.

Figure 3 shows the relationship of SBSLS AUDPC in 2009 with the severity of this disease in 2008 under field

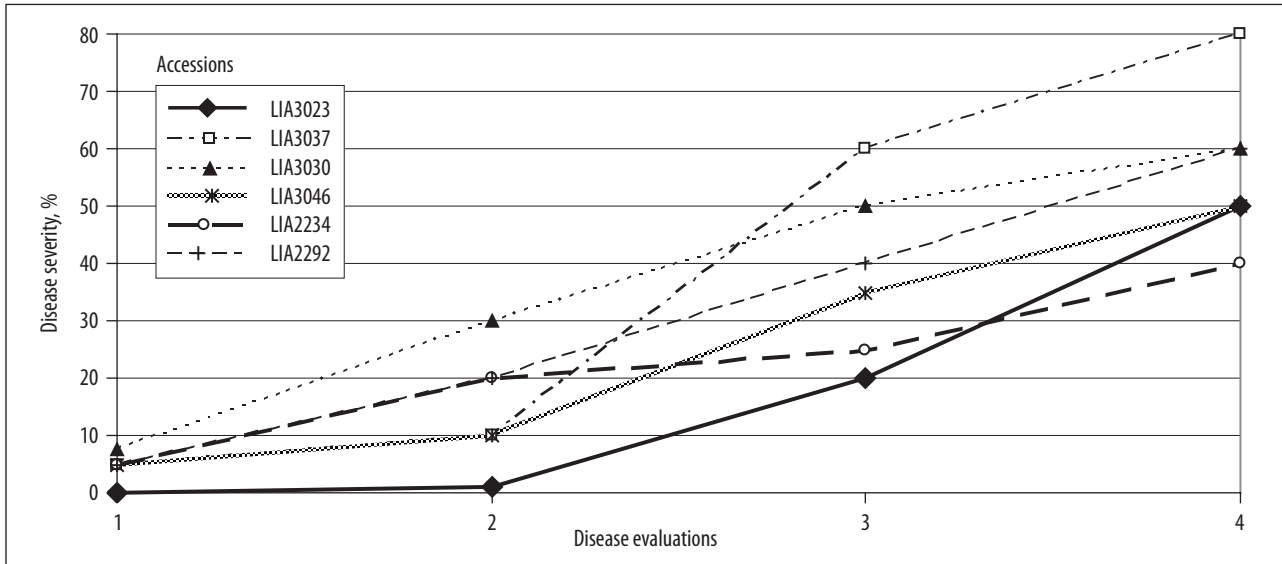


Fig. 1. Development of spring black stem and leaf spots on lucerne accessions possessing different resistance, 2009

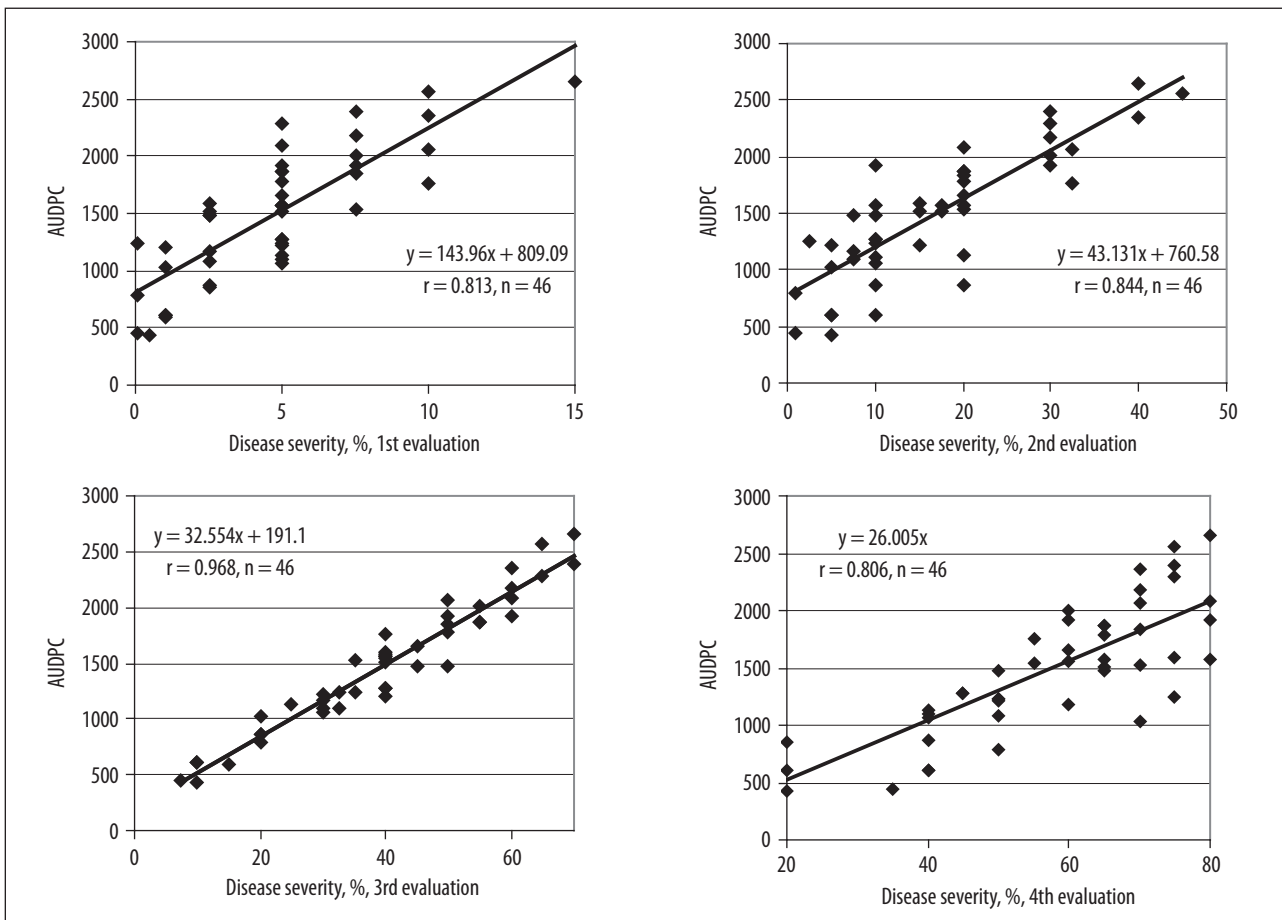


Fig. 2. Correlation of spring black stem and leaf spot area under disease progression curve with disease severity at different disease development moments in 2009

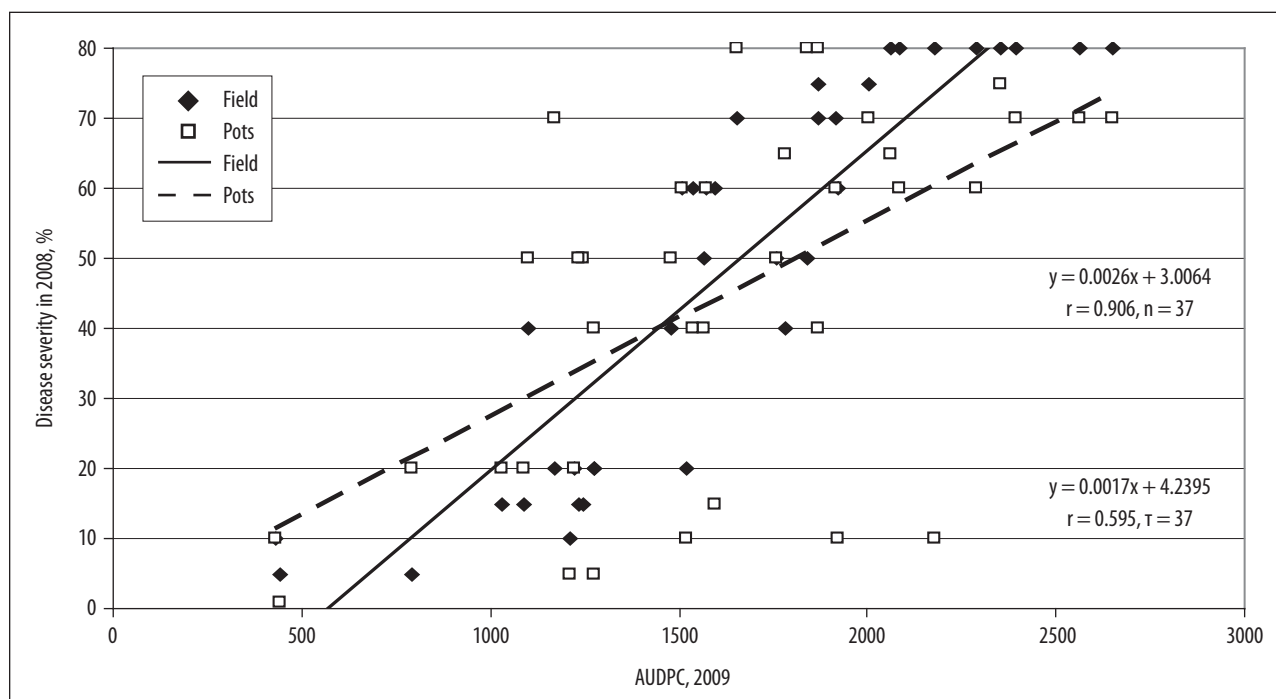


Fig. 3. Relationship of spring black stem and leaf spot (SBSLS) area under disease progression curve in 2009 with SBSLS severity in 2008 in field and pot experiments

and pot conditions. SBSLS AUDPC in 2009 strongly correlated ($r = 0.906^{**}$) with DS under field conditions in 2008, whereas SBSLS AUDPC correlated weaker ($r = 0.589^{**}$) with DS under pot conditions in 2008. A strong correlation among the severity of SBSLS in 2008 and AUDPC in 2009 shows a convenient possibility to evaluate SBSLS resistance of lucerne accessions under a wide range of environments. A medium correlation between SBSLS severity in pots and SBSLS AUDPC in 2009 shows less possibilities for a fast evaluation of lucerne accessions. However, a detailed analysis of the distribution of reaction to SBSLS between both environments suggests that the lower correlation was due rather to the distant reaction of several accessions from the main tendency than that of the total weaker relation.

A broad range of reactions was determined between SBSLS AUDPC and other traits (Table). The highest negative impact of SBSLS was found for pod setting ($r = -0.716^{**}$), overwintering ($r = -0.584^*$), crop rankness ($r = -0.544^*$), amount of grass yield ($r = -0.576^*$) and stem thickness ($r = -0.530^*$). Some traits positively correlated with SBSLS AUDPC. The correlation ($r = 0.702^*$) was strongly positive with resistance to *Sclerotinia* stems and crown rot; medium correlations were determined with the beginning of flowering ($r = 0.544^*$) and inflorescence shape ($r = 0.547^*$). In general, the converging higher SBSLS AUDPC more or less depressed the development of lucerne.

The lucerne accessions distinctly differed by resistance to SBSLS. Disease development was more or less evenly progressive in the genotypes. There were no genotypes with a highly effective resistance; therefore, the accessions possessed partial resistance. Thus, breeding for partial resistance to SBSLS is likely to be a significant goal. Also, this

type of resistance first of all provides long-term resistance (Keller et al., 2000). The quantitative nature of the resistance means that it is more difficult to identify than the race-specific resistance, but it may be apparent as a relatively low disease severity under a high disease pressure.

There are many factors influencing partial resistance to pathogens. Differences in the germination rates of pathogen spores on plant tissues are most likely the intrinsic properties of lucerne genotypes. Availability of carbohydrates and amino acids on leaves of different lucerne genotypes may influenced spore germination. The morphological peculiarities of cuticle are also important as the pathogen penetrates through it (Castell-Miller et al., 2007). Antimicrobial compounds present on leaves can also influence spore germination. Detectable levels of the phytoalexin medicarpin and sativan, a related isoflavonoid, were observed in detached lucerne leaves as early as 8 hours with *P. medicaginis*. The level of resistance was closely related with secreted concentrations of these compounds (Blount et al., 1992). Hydrogen peroxide and related compounds were one of the details of the defence mechanism against *P. medicaginis* infection in *M. truncatula* (Djebali et al., 2007). These and many other factors delay and decrease such pathogen development and spread the events such as spore germination, successful penetration through the cuticle, later development and its rate, the number and size of pycnidia, the number of viable spores per pycnidia, etc.

The use of field scores alone to classify partial resistance may be unreliable. A cultivar may have a low disease severity either because it has a high partial resistance or because weather conditions are unfavourable for disease development (Barbetti, 1991). Also, the amount of the

Table. Coefficients of correlation (r) between area under disease progression curve of spring black stem and leaf spots and other traits of lucerne in 2009

No.	Trait	r
1	SBSLS severity in pots, 2008	0.589**
2	SBSLS severity in field, 2008	0.906**
3	Severity of SBSLS-1, 2009	0.813**
4	Severity of SBSLS-2, 2009	0.844**
5	Severity of SBSLS-3, 2009	0.968**
6	Severity of SBSLS-4, 2009	0.806**
7	Overwintering	-0.584*
8	Resistance to SSCr	0.702*
9	Spring regrowth	-0.09
10	Crop equality	-0.04
11	Crop rankness	-0.544*
12	Amount of leaves	-0.346*
13	Amount of grass yield	-0.576**
14	Stem density	-0.454*
15	Stem thickness	-0.530**
16	Stem colour	0.391*
17	Growth habit	0.175
18	Plant height at bud stage	-0.238*
19	Plant height at flowering	-0.290*
20	Dry mass % of 1st grass	-0.314*
21	Dry mass % of 2nd grass	-0.311*
22	N% in dry mass of 1st grass	-0.837**
23	Length of central leaflet	-0.278
24	Width of central leaflet	-0.262
25	Chlorophyll cons-I	-0.281
26	Chlorophyll cons-II	-0.375*
27	Chlorophyll cons-III	-0.217
28	Beginning of flowering	0.544*
29	Flowers colour	0.309**
30	Inflorescence shape	0.547*
31	Amount of inflorescences	-0.292
32	Pod setting	-0.716**

* Significant at $P < 0.05$; ** $P < 0.01$ probability level.

available inoculum and infection by other pathogens have a high impact. AUDPC was highly correlated with DS during all evaluations. Investigation of AUDPC is time-consuming and laborious, so a high correlation implies that breeders may be able to access their material by single scoring at an appropriate time. Results show that AUDPC values differed at the same DS, especially when the most resistant accessions were compared. Therefore, as partial resistance dominates, it is better to assess lucerne breeding nurseries several times during the vegetation period. On the other hand, simultaneously also the other diseases and traits are assessed, so actually not much time is spent on SBSLS. SBSLS severity in autumn 2008 strongly correlated with SBSLS AUDPC (Fig. 3). One of the reasons was similar weather conditions in 2008 autumn and 2009 June–July, because this pathogen requires wet and cool weather for a strong infection and spread (Barbetti, 1991). This shows the possibility to assess the nurseries by resistance in the year of establishment. Lucerne is a cross-pollinating plant; therefore, accessions in seed production nurseries must be isolated. This factor cannot be avoided during sowing. But some procedures

of removing the undesirable accessions can take place if the resistance of accessions is evaluated before flowering and partial cross pollination among accessions. This could help to accelerate resistance breeding through a decreased flow of inappropriate genes. The medium correlation of SBSLS severity in pots with SBSLS AUDPC also implies the possibility to select resistant material. However, this screening is more appropriate for the evaluation of accessions for generating new cross combinations. The main disadvantage of this method is a relatively high cost of accession testing. A weaker correlation could be due to a lower variability of *P. medicaginis* genotypes and a much shorter period of their adaptation to host plants (Ellwood et al., 2006; Gray et al., 1990; Rodriguez, 2005).

Analysis of correlation of SBSLS AUDPC with other traits showed the mainly negative impact of the disease on lucerne productivity. Some traits that correlated positively can be explained, too. A strong positive correlation ($r = 0.702^*$) with resistance to SSCr should be associated with a lower resistance of plants infected with *P. medicaginis* to SSCr. Lucerne accessions were heavily infected by *P. medicaginis* in autumn 2008. Studies of Rodriguez and Leath (1992) showed a highly negative impact of *P. medicaginis* on the survival of lucerne due to rots of crown and roots.

A positive correlation with the beginning of flowering shows the susceptibility of later developed accessions. The inflorescence shape shows a tendency that more agronomically advanced accessions have a lower resistance level.

The susceptibility showed the strongest negative influence on pod setting and subsequently on seed yield. This relation is one of the reasons why in Lithuania lucerne seed yields are so low in wet years (Šlepetyš, 2004). A similar correlation was determined in many lucerne production areas (Hwang et al., 2006; Leyronas et al., 2004; Barbetti, Nichols, 1991). The negative impact of susceptibility to *P. medicaginis* decreased overwintering ($r = -0.584^*$). Lucerne is a perennial plant that should maintain an even crop stand; therefore, decreasing plant density negatively affects the profitable crop age. Also, the negative reaction to *P. medicaginis* was observed in the case of crop rankness, the amount of leaves and grass yield, stem density and thickness ($r = -0.346^* - 0.576^{**}$). These observations agree with data across the world (Babnik, 1995; Campbell, Duthie, 1990; Fonseca et al., 1999; Nutter et al., 2002).

Beside the obvious physical damage, SBSLS in lucerne affect leaf metabolism, which in turn affects its productivity and quality. Recent studies demonstrated that foliar diseases reduced crude proteins in leaves (Babnik, 1995; Hwang et al., 2006). Our results also showed a strong negative ($r = -0.837^{**}$) correlation with the damage of nitrogen level in the dry mass of the 1st grass. One of the reasons why proteins decrease due to diseases is defoliation (Guan, Nutter, 2002; Nutter et al., 2002). The negative effect of the disease on chlorophyll concentration ($r = -0.217 - 0.375^*$) also explains the decrease of forage value (Guan, Nutter, 2002;

Hwang et al., 2006). Accessions more severely damaged by *P. medicaginis* produce higher concentrations of phytoestrogens. These substances can negatively affect the health of livestock (Barbetti, 2007; Weber et al., 2004).

The broad range of negative effects of *P. medicaginis* on lucerne traits indicates an imperative of exhaustive lucerne resistance breeding in Lithuania.

CONCLUSIONS

1. The lucerne accessions (cultivars and populations) distinctly differed by resistance to spring black stem and leaf spots (SBSLS). Disease development was more or less evenly progressive in the genotypes. Therefore, the accessions showed partial resistance.

2. In the most resistant accessions, the values of the SBSLS area under the disease curve (AUDPC) ranged within 429–868, whereas the most susceptible ones were evaluated by the AUDPC values of 2060–2650.

3. The correlation between disease severity (DS) and AUDPC was strong ($r = 0.806^{**}$ – 0.968^{**}) in all assessments. The variability of AUDPC values was higher for more resistant accessions than for more susceptible ones in one DS group.

4. SBSLS AUDPC in 2009 strongly correlated ($r = 0.906^{**}$) with DS under field conditions in 2008, whereas SBSLS AUDPC correlated weaker ($r = 0.589^{**}$) with DS under pot conditions in 2008.

5. A broad range of reactions was determined between SBSLS AUDPC and other traits. The highest negative impact of SBSLS was found for pod setting ($r = -0.716^{**}$), overwintering ($r = -0.584^{*}$), crop rankness ($r = -0.544^{*}$), grass yield ($r = -0.576^{*}$) and stem thickness ($r = -0.530^{*}$).

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GRYBINĖS LIGOS, SUKELIAMOS PHOMA MEDICAGINIS, ĮTAKA LIUCERNAI (MEDICAGO SPP.)

Santrauka

Vegetaciniuose puoduose tirti 37 *M. sativa*, *M. varia* ir *M. falcata* (*Medicago* spp.), o lauko bandymuose 46 *Medicago* spp. pavyzdžiai (veislės ir populiacijos) pagal atsparumą grybinės ligos sukėlėjui *Phoma medicaginis* natūraliame infekciniame fone 2008–2009 m. Tirtų liucernos pavyzdžių atsparumas askochitozei priklausė nuo įvairaus lygio dalinio atsparumo. Atspariausi pavyzdžiai buvo įvertinti ligos progreso sezono metu (AUDPC) reikšmėmis 429–868, o jautriausi – 2060–2650. Askochitozės intensyvumo reikšmės visais 4-iais ligos vertinimais stipriai koreliavo ($r = 0.806^{**}$ – 0.968^{**}) su AUDPC reikšmėmis. Ligos intensyvumas lauko sąlygomis 2008 m. stipriai koreliavo ($r = 0.906^{**}$) su AUDPC reikšmėmis 2009 m. Tačiau ligos intensyvumas vegetacinių puodų sąlygomis su AUDPC reikšmėmis koreliavo silpniau ($r = 0.589^{**}$). Jautrumas askochitozei neigiamai stipriai ir vidutiniškai koreliavo su ankštelių užmezgimu ($r = -0.716^{**}$), peržiemojimu, ($r = -0.584^{*}$), augalų vešlumu ($r = -0.544^{*}$), žolės kiekiu ($r = -0.576^{*}$) ir stiebų storiumi ($r = -0.530^{*}$).

Raktažodžiai: *Medicago*, *Phoma medicaginis*, atsparumas, AUDPC