Acta Sci. Pol. Hortorum Cultus, 18(1) 2019, 47–56

www.acta.media.pl ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2019.1.5

ORIGINAL PAPER

Accepted: 6.06.2018

SUSCEPTIBILITY OF LEMON BALM (*Melissa officinalis* L.) VARIETIES TO SEPTORIA LEAF SPOT (*Septoria melissae* Desm.) IN HUNGARY

Gergő Kovács[1]✉, Éva Zámbori-Németh[1], Géza Nagy[2]

[1] Department of Medicinal and Aromatic Plants, Szent István University, H-1118 Budapest, Villányi 29-43, Hungary

[2] Directorate of Plant Protection, Soil Conservation and Agri-environment, National Food Chain Safety Office, H-1118 Budapest, Budaörsi 141-145, Hungary

ABSTRACT

Lemon balm (*Melissa officinalis* L.) is a widely cultivated plant in Europe. *Septoria melissae* Desm. is the most important pathogen of lemon balm crops, which may cause serious yield loss by the severe leaf fall. As chemical control of the pathogen is restricted, alternative plant protection methods, like the use of tolerant varieties, should be taken under consideration. The goal of our work was to evaluate the susceptibility of three lemon balm varieties to Septoria leaf spot in field. The trials were carried out in 2016 and 2017 in Budapest-Soroksár, Hungary. Dynamics of infection showed characteristic sharp increase from the beginning of August in both years. The lowest infection levels were observed in cultivar 'Lemona'. The disease incidence in the middle of August was 19% in the first year and 59% in the second year, while these values were over 40% and 70% in the other cultivars 'Soroksári' and 'Quedlinburger Niederliegende', respectively. The manifestation of symptomps was also significantly lighter on the 'Lemona' plants and they had the highest ratio of healthy leaves (81%) compared to the other cultivars. According to the data, weather conditions might modify the range of the differences among the cultivars. It was concluded that appropriate selection of varieties could be an effective and enviromental friendly plant protection method in the practice of lemon balm cultivation.

Key words: *Melissa officinalis*, *Septoria melissae*, Septoria leafspot, cultivars, susceptibility

INTRODUCTION

Lemon balm (*Melissa officinalis* L.) is a popular perennial medicinal and aromatic plant (MAP) belonging to the *Lamiaceae* family. Shoots and leaves of the plant are popular ingredients of different herbal tranquilizers or teas and could be used as edible decoration as well. Lemon balm is also a traditional folk medicine applied to cure nervousness, gastrointestinal disorders and against insomnia [Engel et al. 2016]. The essential oil and extracts from lemon balm are the object of increasing interest due to their potential pharmaceutical usage. *In vitro* investigations carried out by Schnitzler et al. [2008] indicated that the essential oil of the plant inhibits the replication of *Herpes simplex virus*. Furthermore, rosmarinic acid, an important component of lemon balm, showed neuroprotective effect in clinical investigations [Ramanauskiene et al. 2016].

Cultivation of the plant is common in several European countries like Germany, Poland and also in Hungary [Seidler-Łożykowska et al. 2013, Bernáth and Zámbori-Németh 2015, Russo 2017]. According to the literature, several pathogens could infect lemon

✉ kovacs.gergo.phd@gmail.com

balm i.e. *Septoria melissae* Desm., *Oidium* spp., *Alternaria* spp., *Puccina menthae* Pers. Among all pathogens, *S. melissae* is responsible for the greatest damage to the plants [Nagy and Horváth 2010, Jadczak and Pizoń 2017, Wielgusz and Seidler-Łożykowska 2017]. In Hungary, the first symptoms usually appear on the older leaves in May [Nagy 2002]. Spots are angular or irregular, dark brown or greyish in color, often with a purplish margin [Nagy and Horváth 2010, Hoppe 2013, Kowalska et al. 2014]. The disease frequently leads to serious yield loss due to severe leaf fall. Even a less severe form of the disease may influence the quality of the drug by decreasing the yield of essential oil and altering its composition [Aulerio et al. 1995, Kowalska et al. 2014].

Plant protection of MAPs is often inadequate in practice. The possibility of disease management by chemicals is in most cases restricted due to strict regulation of the maximum allowed residue levels in herbal products [Kowalska et al. 2014, Bernáth and Zámbori-Németh 2015]. Besides, there are only a few pesticides authorized for the protection of MAPs including lemon balm [Ocskó et al. 2017]. Considering above, the sustainable and efficient protection of MAPs seems to be quite difficult. Using tolerant/resistant genotypes is one of the most advantageous alternative crop protection method compared to the use of chemicals [Lynch et al. 2017]. However, there is a lack of information about the intraspecific variability of lemon balm concerning resistance against phytopathogens. The only available reference from Meyers [2007] reports a moderate resistance of the cultivar 'Citronella' against powdery mildew disease. Susceptibility of the intraspecific varieties to *S. melissae* is not known or has not been published yet.

The goal of this study was to evaluate the susceptibility of three lemon balm cultivars to Septoria leaf spot under field conditions.

MATERIAL AND METHODS

Plant material

The study was carried out in 2016 and 2017 at the Experimental and Research Farm of the Szent István University at Budapest-Soroksár (47°24'08.7''N, 19°09'03.9''E), Hungary. The experimental field has sandy soil with slight acidic (6.21) pH value, and high phosphorus (1330 mg kg^{-1}), moderate potassium (463 mg kg^{-1}) and low nitrogen (36 mg kg^{-1}) content. The humus content is 0.76%.

The weather conditons were different in the two growing seasons. Distribution of rainfall was uneven in 2017 and less precipitation (by 140 mm) was measured compared to more humid vegetation period of 2016 (Tab. 1). Therefore, plots were irrigated with 10–15 mm water three times a week during the arid period from the begining of July in 2017.

The small plot (2 m × 0.4 m) trial has been carried out in five replicates (5 plots/cultivar) in 2016 and in six replicates (6 plots/cultivar) in 2017. Annual plants were investigated in both years. The seed material of the cultivars 'Lemona' and 'Quedlinburger Niederliegende' was purchased from a commercial company (Jelitto Staudensamen GmbH). Accession 'Soroksári' was retrieved from the gene bank of the Department of Medicinal and Aromatic Plants, Szent István University. Seedlings were grown in greenhouse conditions and two-month-old plants were planted out on the 31th of May in 2016 and on the 7th of June in 2017. Plant spacing was 0.9 m × 0.4 m. Afterwards, the plants received 2 mm water three times a week for a fortnight to enhance rooting. Weed control was carried out mechanically. Harvesting of the plants was carried out on the 15th of September in 2016 and on the 31th of August in 2017.

Identification of the pathogen

Identification was carried out by symptomological observations and morphological investigations of the fungal isolates collected from the field. The published descriptions [e.g. Nagy et Horváth 2010, Hoppe 2013] are in accordance with the symptomps observed on leaves (Fig. 1).

Infected leaves were collected from the experimental plots for morphological investiagtions. The leaves were placed into moist chamber for 24 hours. Conidia from conidial exudates appeared on the surface of the leafspots, were measured for size and number of septums by Nikon Eclipse 50i cytosol microscope. Isolates were isolated on malt extract agar medium. Morphological characteristics of the isolates were in accordance with data published by Brandenburger [1985] and Verkley et al. [2013].

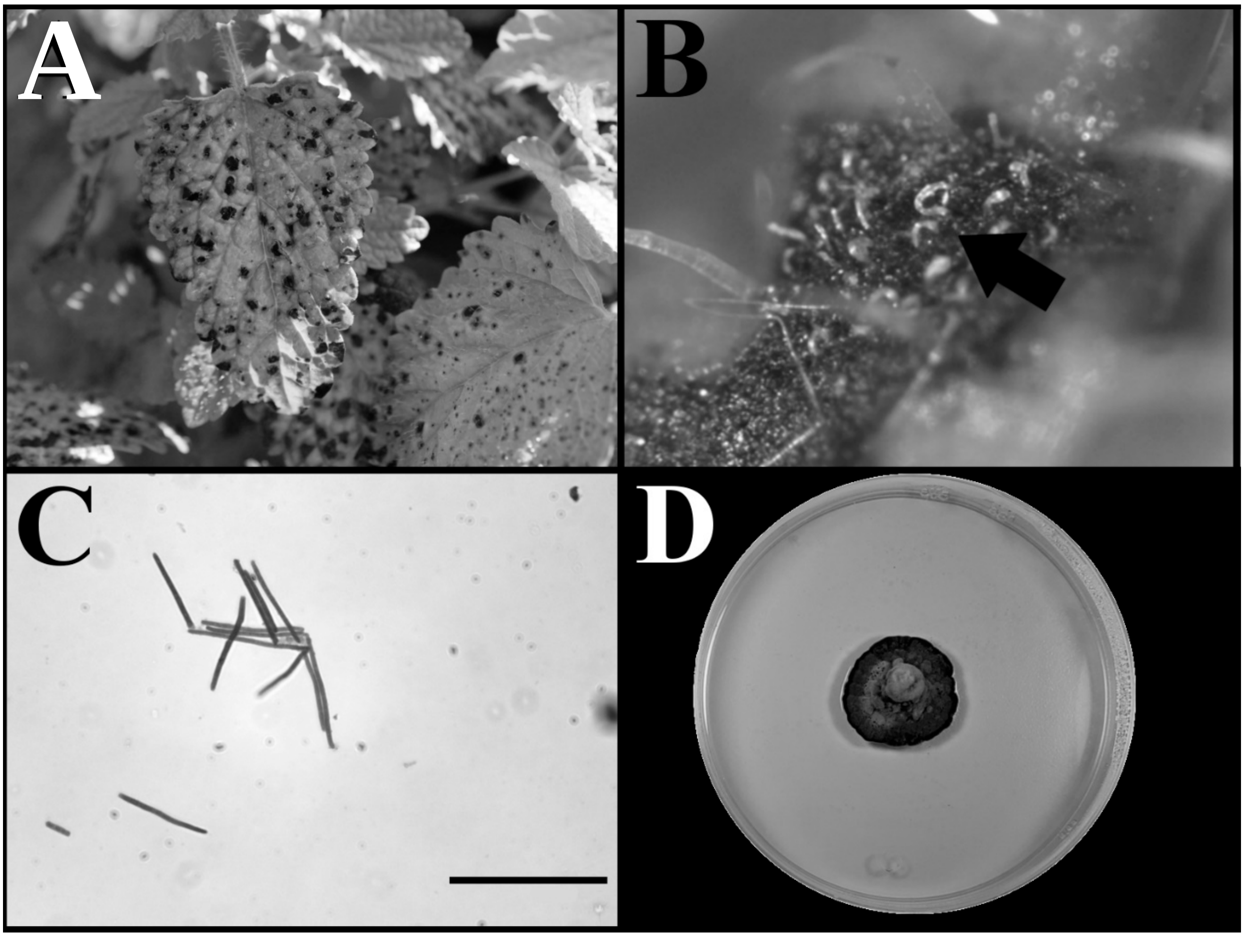
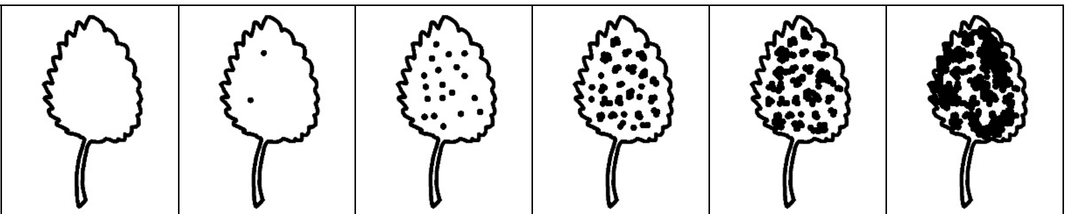


Fig. 1. Morphological patterns of *Septoria melissae* Desm. A – symptomps on the leaves. B – conidial exudates on the surface of leafspot, after incubation in wet chamber. C – conidia from the exudates – bar 50 µm. D – colony of the fungus on malt extract agar



Infection category	0	1	2	3	4	5
Necrotized leaf surface*	0%	1–5%	6–25%	26–50%	51–75%	>75%

*Share of necrotized area to the whole leaf surface

Fig. 2. Infection categories based on leaf symptoms

Disease assessment

Assessments of disease level were carried out weekly from June to September in both years. In the first year, 25 randomly selected leaves were evaluated on every plant. In the second year, all of the leaves of 2–4 fully developed shoots were evaluated on each plant (in total about 25–45 leaves/plant).

Infection rate has been defined by the following parameters: the disease incidence (DI) was calculated by the ratio of infected leaves and totally evaluated ones in each plants and expressed in percentage. The disease severity index (DSI) was calculated by the formula of Towsend and Heuberger [Gartner 1971] based on the size of necrotized area compared to the total surface of the leaf. The ratio of the leaves in different infection categories was also compared. Evaluated leaves were classified into the following five infection categories: 0: healty leaf, 1: 1–5% necrotized leaf area, 2: 6–25% necrotized leaf area, 3: 26–50% necrotized leaf area, 4: 51–75% necrotized leaf area, 5: >75% necrotized leaf area (Fig. 2).

Statistical analysis

A statistical analysis was carried out with data of the last three assessments of both vegetation periods assuring the most representative results. Data were analyzed by the IBM SPSS Statistics 22 software. Multivariate ANOVA (MANOVA), Chi square and z-test tests were applied to evaluate the significant differences among the susceptibility of investigated cultivars.

Normality of residuals was tested according to the Saphiro-Wilk and Kolgomorov-Smirnov tests. If the normality could not be justified by mentioned analyses, it was verified by the skewness and kurtosis. Homogeneity of variances was tested by the Levene's method. If the homogenity assumption was not violated, Tukey *post hoc* test was used to group the genotypes. Otherwise, the separation was made by the Games-Howell test.

The comparison of the count of leaves in different infection categories was carried out by the Pearson Chi square method. Separation of different groups in each category was made by the z-test.

RESULTS AND DISCUSSION

We detected a characteristic dynamics of the development of the disease on lemon balm. The first symptomps could be observed on leaves as early as the second week after planting in 2016. In the following year, leafspots appeared later, one month after the estabilishment of the plantation. The infection increased to a remarkable level by the beginning of August in both years (Figs. 3–6), supposedly due to more rainy period at the end of July and the beginning of August (Tab. 1).

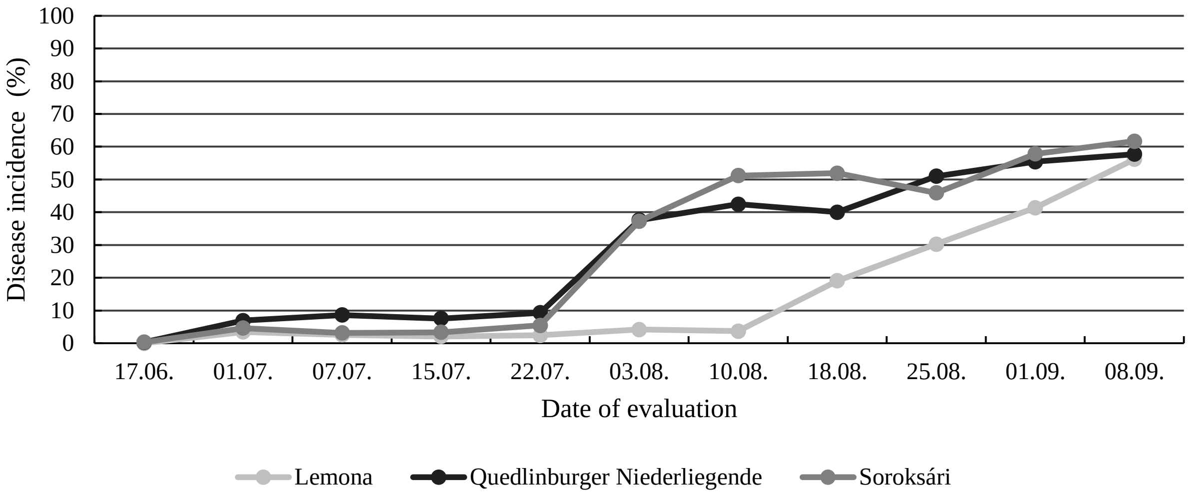


Fig. 3. Disease incidence (DI) of three lemon balm cultivars during the vegetation period of 2016

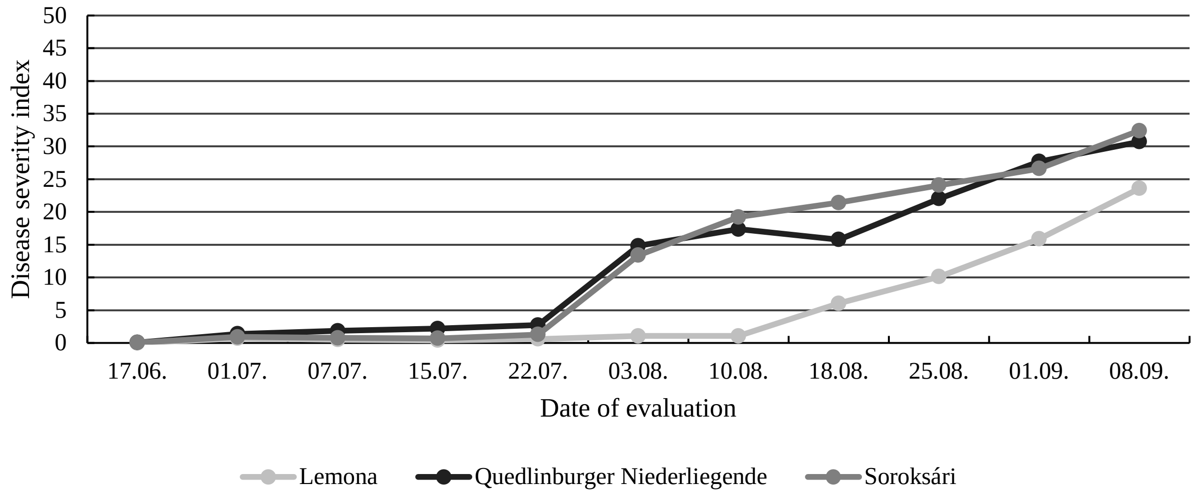


Fig. 4. Disease severity index (DSI) of the three lemon balm cultivars during the vegetation period of 2016

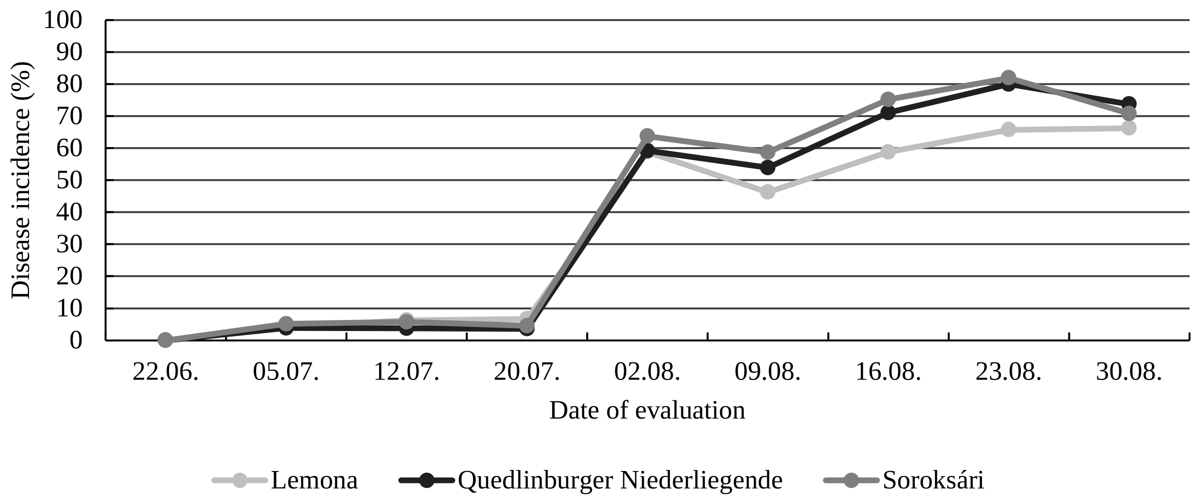


Fig. 5. Disease incidence (DI) of the three cultivars during the vegetation period of 2017

Concerning disease severity, differences between the experimental years were also remarkable. Both DI and DSI were higher in the second year, despite of more humid weather conditions (Figs. 5–6). Most likely, the applied irrigation and resulting higher humidity in the plant stand in the second year facilitated the multiplication and spread of the pathogen.

Remarkable differences among the cultivars in susceptibility to Septoria leaf spot could be observed in both years, however the strength of the effect was influenced by weather conditions.

In 2016, the lowest disease incidence was observed for cultivar 'Lemona'. By the beginning of August, it reached only 4.2%, however, even at the end of summer, the disease incidence remained 14–16% lower compared to the other cultivars (Fig. 3). The cultivars 'Soroksári' and 'Quedlinburger Niederliegende' did not differ significantly from each other in this respect (except from disease incidence evaluated on 18th August) (Fig. 7). A similar tendency could be observed for disease severity index (DSI). Manifestation of the symptomps was significantly lighter (the index was lower by 8–18) on 'Lemona' plants than on the leaves of other genotypes during the vegetation (Fig. 3). Lower disease levels were observed on the plants of 'Lemona' genotype, which were justified by the results of statistical analysis (Fig. 7).

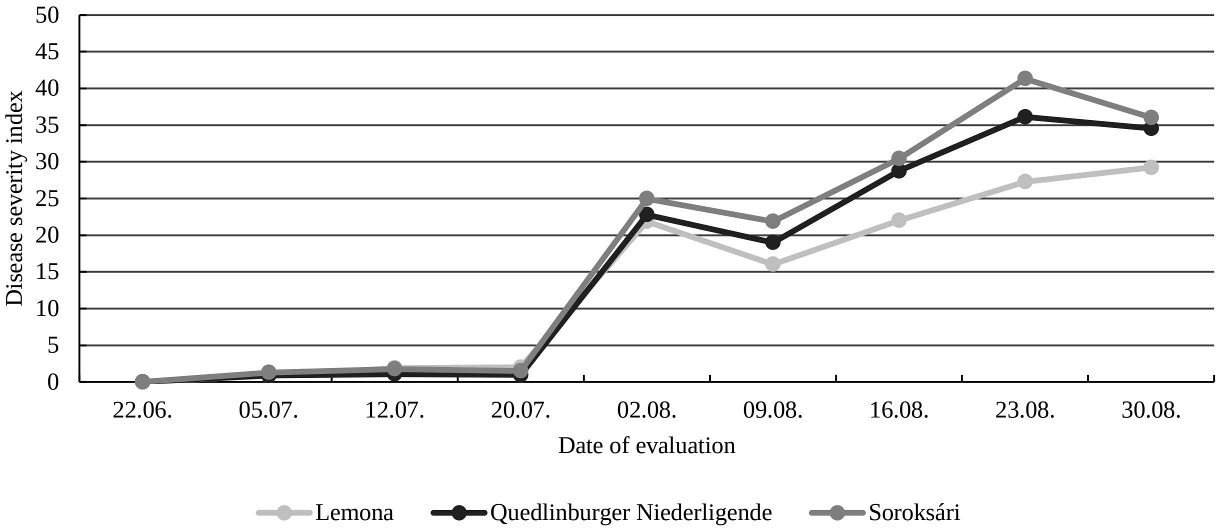


Fig. 6. Disease severity index (DSI) of the three cultivars during the vegetation period of 2017

Table 1. Precipitation and temperature data of the experimental site in both vegetation periods (2016 and 2017) from June to September

2016											
Month	June			July			August			September	
10-day period	I	II	III	I	II	III	I	II	III	I	II
Precipitation (mm)	26.2	36.2	27.4	1.6	89.4	59.4	15.2	22.2	66.6	21	43.2
Temperature (°C)	18.1	18.92	23.48	20.97	20.08	22.791	20.62	18.31	19.764	19.24	18.69
2017											
Month	June			July			August			September	
10-day period	I	II	III	I	II	III	I	II	III	I	II
Precipitation (mm)	0.4	3.4	30	44.6	6.2	42.8	34.4	27.6	1	26.8	50
Temperature (°C)	20.42	20.17	23.17	22.17	20.62	21.5	25.33	21.83	18.827	16.85	15.133

Table 2. Number of leaves in different infection categories at the selected three assessments before harvest in 2016

Date of measurement			18.08.			01.09.			08.09.		
		Cultivar	L	Q	S	L	Q	S	L	Q	S
Infection categories	0	Count	506a	375b	299c	368a	278b	262b	274a	264a	230a
		Expected Count	394.4	394.4	391.2	303.2	303.2	301.7	257.5	256.7	253.8
	1	Count	50a	49a	57a	35a	40a	84b	57a	30b	40a, b
		Expected Count	52.1	52.1	51.7	53.1	53.1	52.8	42.6	42.4	42.0
	2	Count	68a	159b	179b	212a	153b	135b	193a	174a	172a
		Expected Count	135.7	135.7	134.6	166.9	166.9	166.1	180.7	180.1	178.1
	3	Count	1a	42b	85c	10a	110b	96b	95a	68a	77a
		Expected Count	42.8	42.8	42.4	72.1	72.1	71.8	80.5	80.2	79.3
	4	Count	0	0	0	2a	43b	36b	6a	64b	70b
		Expected Count	0.0	0.0	0.0	27.0	27.0	26.9	46.9	46.8	46.3
	5	Count	0	0	0	0a	3a. b	11b	0a	23b	27b
		Expected Count	0.0	0.0	0.0	4.7	4.7	4.7	16.8	16.7	16.5
Total		Count	625	625	620	627	627	624	625	623	616
		Expected Count	625.0	625.0	620.0	627.0	627.0	624.0	625.0	623.0	616.0

L – ’Lemona’; Q – ’Quedlinburger Niederliegende’; S – ’Soroksári’; a, b, c – letters refer to significant differences according to the z-test among the cultivars at each assessment separately

Table 3. Number of leaves in the different infection categories at the last three assessments before harvest in 2017

Date of measurement			16.08.			23.08.			30.08.		
		Cultivar	L	Q	S	L	Q	S	L	Q	S
Infection categories	0	Count	441a	301b	240c	358a	165b	176b	311a	272a. b	182b
		Expected Count	352.6	312.6	316.8	258.7	206.3	234.0	278.3	280.1	206.5
	1	Count	131a	116a	179b	123a	104a	96a	87a	85a	75a
		Expected Count	153.0	135.6	137.4	119.5	95.3	108.1	89.9	90.4	66.7
	2	Count	451a. b	427b	373a	414a	328a. b	325b	325a	267b	243a
		Expected Count	449.2	398.2	403.6	394.9	315.0	357.2	303.8	305.8	225.4
	3	Count	51a	96b	147c	135a	164b	233c	177a	188a	122a
		Expected Count	105.6	93.6	94.9	196.9	157.0	178.1	177.2	178.3	131.5
	4	Count	0a	9b	25c	17a	65b	91b	19a	90b	55b
		Expected Count	12.2	10.8	11.0	64.0	51.1	57.9	59.7	60.1	44.3
	5	Count	0a	3a	1a	1a	10b	27c	0a	23b	5c
		Expected Count	1.4	1.3	1.3	14.1	11.2	12.7	10.2	10.3	7.6
Total		Count	1074	952	965	1048	836	948	919	925	682
		Expected Count	1074.0	952.0	965.0	1048.0	836.0	948.0	919.0	925.0	682.0

L – ‘Lemona’; Q – ‘Quedlinburger Niederliegende’; S – ‘Soroksári’; a, b, c – letters refer to significant differences according to the z-test among the cultivars at each assessment separately

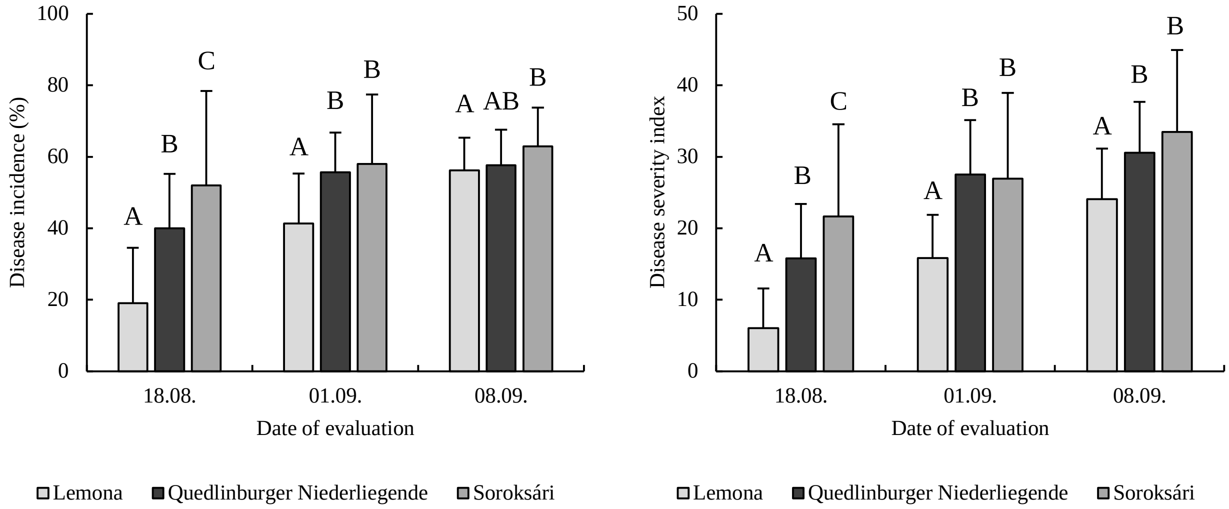


Fig. 7. Results of disease incidence (DI) and disease severity index (DSI) of the investigated cultivars at the selected three assessments in 2016. A, B, C letters refer to significant differences based on the Tukey and the Games-Howell *post hoc* tests among the cultivars at each assessment separately

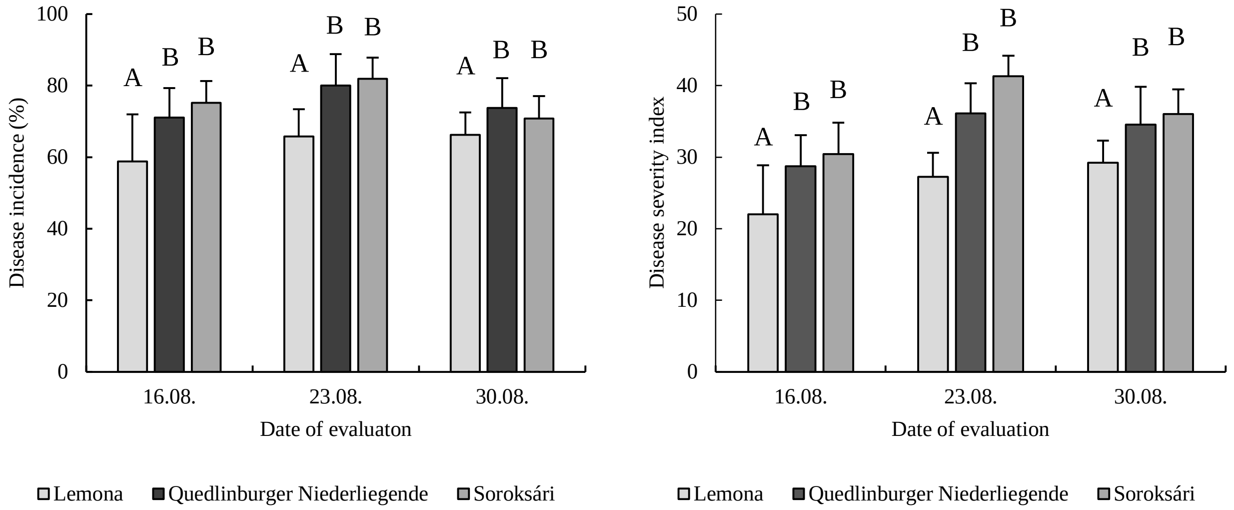


Fig. 8. Results of disease incidence (DI) and disease severity index (DSI) at the last three assessments in 2017. A, B, C letters refer to significant differences based on the Tukey and the Games-Howell *post hoc* tests among the cultivars at each assessment separately

Differences among cultivars concerning the number of leaves classified in different infection categories were also justified (Tab. 2). Cultivar 'Lemona' had the highest ratio of healthy leaves (81%) compared to the other two genotypes by the middle August. On the other hand, more severe types of symptoms (categories 4 and 5) occurred in very low abundance (<1%) in these plots. The fungus appeared earlier and caused larger infected leaf area on the plants of the other two cultivars than on 'Lemona'.

A higher infection level in general could be observed on the experimental plots in 2017. Differences among varieties could be detected from the beginning of August both in DI and DSI. The ratio of infected leaves (DI) was lower by 10% in average in the plots of 'Lemona' compared to the other cultivars (Fig. 5).

'Lemona' revealed better results in the parameter of necrotized leaf surface (DSI) as well. The extent of DSI measured at the end of summer in 2017 was 16–23% larger on the varieties 'Soroksári' and 'Quedlinburger Niederliegende' than on 'Lemona' (Fig. 6). According to the statistical analysis, 'Lemona' cultivar was significantly different from other genotypes at the last three assessments (16.08., 22.08., 30.08.) before harvest (Fig. 8).

Based on the values of different infection categories, the leafspot development on the plants of 'Soroksári' genotype was faster than on the other cultivars (Tab. 3). Starting from the 16th of August, leaves with infection categories 4 and 5 could be observed on these plants with at least 40–170% higher frequency than on the plants of 'Lemona' and 'Quedlinburger Niederliegende'. At the last assessment, slightly lower values could be detected again, presumably, because the most seriously infected leaves were already fallen down.

Our results show that the intraspecific varieties of lemon balm may have significant influence on the development of Septoria leafspot disease symptoms. Out of the investigated cultivars, 'Lemona' proved to be the least susceptible to *Septoria melissae* Desm. On the other hand, the fastest symptom development and the highest infection levels were observed in the plots of 'Soroksári' cultivar. It could be concluded, that weather conditions during the vegetation period may influence not only the disease development in general, but also modify the strength of the effect of the cultivars on the appearance of symptoms. Under arid conditions, these differences are less considerable.

These results indicate that the selection of varieties could be an effective and environmental friendly plant protection method in the practice of lemon balm cultivation. Nevertheless, further studies are needed to detect the physiological/biochemical background of the variable susceptibility of the intraspecific accessions.

CONCLUSIONS

1. There is a considerable intraspecific variability of *Melissa officinalis* concerning the susceptibility for *Septoria melissae* disease.

2. Out of the investigated three cultivars, 'Lemona' proved to be the least susceptible to *Septoria melissae* Desm., while the fastest symptom development and the highest infection levels were detected in 'Soroksári' cultivar.

3. Weather conditions may influence not only the appearance and development of the fugus, but also differences in the manifestation of symptoms among cultivars.

4. Growing the disease-tolerant varieties would be an important and effective tool in the cultivation practice of medicinal and aromatic plants.

ACKNOWLEDGEMENTS

This research was supported by the Higher Education Institutional Excellence Program (1783-3/2018/FEKUTSTRAT) awarded by the Ministry of Human Capacities within the framework of plant breeding and plant protection researches of Szent István University.

REFERENCES

Auleiro, A.Z., Zambonelli, A., Bianchi, A., Albasini, A. (1995). Micromorphological and chemical investigations into the effects of fungal diseases on *Melissa officinalis* L., *Mentha piperita* L. and *Salvia officinalis* L. J. Phytopathol., 143(3), 179–183.

Bernáth, J., Zámboriné Németh, É. (2015). Gyógynövény kultúrák magyarországi növényvédelmének időszerű kérdései [Up to date aspects of the plant protection in the Hungarian medicinal plant production]. Növényvédelem, 51(1), 25–36.

Brandenburger, W. (1985). Parasitische Pilze an Gefässpflanzen in Europa. Gustav Fischer Verlag, Stuttgart−New York.

Engel, R., Szabó, K., Abrankó, L., Rendes, K., Füzy, A., Takács, T. (2016). Effect of arbuscular mycorrhizal fungi on the growth and the polyphenol profile of marjoram, lemon balm and marigold. J. Agric. Food Chem., 64(19), 3733−3742. DOI: 10.1021/acs.jafc.6b00408.

Gartner, H. (1971). Versuche zur Bekämpfung von *Botrytis cinerea* (Grauschimmel) als Traubenfäule. Mitt. Klosterneuburg, 21(3), 183–189.

Hoppe, B. (ed.). (2013). Handbuch des Arznei- und Gewürzpflanzenanbau, Band 5. Saluplanta e.V./GFS e.V., Bernburg.

Jadczak, P., Pizoń, K. (2017). Identification of taxa of microscopic fungi occurring on selected herbal plants and possible methods of their elimination. World Sci. News, 69, 1−17.

Kowalska, J., Remlein-Starosta, D., Seidler-Łożykowska, K., Bocianowski, J. (2014). Can *Trichoderma asperellum* [t1] stimulate growth of lemon balm (*Melissa officinalis* l.) in different systems of cultivation? Acta Sci. Pol. Hortorum Cultus, 13(1), 91–102.

Lynch, J.P., Glynn, E., Kildea, S., Spink, J. (2017). Yield and optimum fungicide dose rates for winter wheat (*Triticum aestivum* L.) varieties with contrasting ratings for resistance to *Septoria tritici* blotch. Field Crops Res., 204, 89–100.

Meyers, M. (2007). Lemon balm: An Herb Society of America guide. The Herb Society of America, Kirtland.

Nagy, G. (2002). A szeptóriás betegség kártétele citromfűn [Damage on lemon balm in consequence of Septoria infection]. Növényvédelem, 38(4), 185–187.

Nagy, G., Horváth, A. (2010). Gyógynövények szeptóriás levélfoltosságai Magyarországon [*Septoria* leaf spot diseases of medicinal plant species in Hungary]. Növényvédelem, 46(4), 145–153.

Ocskó, Z., Erdős, Gy., Molnár, J. (2017). Növényvédő szerek, termésnövelő anyagok 2017. Vol. I. Agrixen Kiadó, Budapest, p. 789.

Ramanauskiene, K., Raudonis, R., Majiene, D. (2016). Rosmarinic acid and *Melissa officinalis* extracts differently affect glioblastoma cells. Oxidative Med. Cellular Longev., Article ID 1564257. DOI:10.1155/2016/1564257.

Russo, M. (2017). Effect of shading on leaf yield, plant parameters, and essential oil content of lemon balm (*Melissa officinalis* L.). J. Appl. Res. Med. Arom. Plants, 7, 27–34. http://dx.doi.org/10.1016/j.jarmap.2017.04.003

Schnitzler, P., Schuhmacher, A., Astani, A., Reichling, J. (2008). *Melissa officinalis* oil affects infectivity of enveloped herpesviruses. Phytomedicine, 15, 734–740.

Seidler-Łożykowska, K., Bocianowski, J., Król, D. (2013). The evaluation of the variability of morphological and chemical traitsof the selected lemon balm (*Melissa officinalis* L.) genotypes. Ind. Crops Prod., 49, 515–520.

Verkley, G.J.M., Quaedvlieg, W., Shin, H.D., Crous, P.W. (2013). A new approach to species delimitation in *Septoria*. Stud. Mycol., 75, 213–305.

Wielgusz, K., Seidler-Łożykowska, K. (2017). Fungi colonizing and damaging different parts of some medicinal plants. Herba Pol., 63(2), 18–26.