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ORIGINAL ARTICLE

Response of lemon balm (*Melissa officinalis* L.) accessions to Septoria leaf spot (*Septoria melissae* Desm.) in Hungary

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Abstract

Septoria melissae Desm., the most important pathogen of lemon balm (*Melissa officinalis*) occurs each year on plantations. The fungus may cause serious yield losses in the absence of proper plant protection. Breeding resistant or tolerant cultivars could play an important role in plant protection of medicinal plants. However, only a few descriptions of tolerant varieties of lemon balm are available. The goal of this work was to evaluate the susceptibility of three accessions of *M. officinalis* against the pathogen of Septoria leaf spot under field conditions at Budapest-Soroksár (Hungary) in 2017–2018. Differences in susceptibility of the accessions were observed in both years. The accession of *M. officinalis* subsp. *altissima* proved to be the least susceptible to *Septoria* infection. The frequency of the infected leaves was only 5.1 and 28.1% in 2017 and 2018, respectively. However, the cultivar *M. officinalis* subsp. *officinalis* 'Lorelei' turned out to be the most susceptible to the pathogen with an average infection level of 26.1 and 66.6%, 1.3–6.1 times higher than that of the other accessions in each year, respectively. Development of disease tolerant *M. officinalis* cultivars may be an effective tool in the plant protection of lemon balm.

Keywords: medicinal plants, *Melissa officinalis*, resistance breeding, Septoria leaf spot

Introduction

Lemon balm (*Melissa officinalis* L.) is a popular perennial medicinal and aromatic plant (MAP) belonging to the Lamiaceae family. The leaves are often found to be components of different herbal tranquilizers or teas. Lemon balm is considered as a traditional folk medicine and is used as a mild sedative to cure nervousness and insomnia (Patora *et al.* 2003; Engel *et al.* 2016). The essential oil and extracts of lemon balm are the objects of increasing interest by their potential pharmaceutical usage. *In vitro* investigations carried out by Schnitzler *et al.* (2008) indicated that the essential oil inhibits the replication of *Herpes simplex virus*. The oil is also used in cosmetics (Patora *et al.* 2003). The main compounds of the essential oil are geraniol, neral and citronellal, which give its citrus fragrance (Dawson *et al.* 1988). Rosmarinic acid, the other important component, showed a neuroprotective effect in clinical investigations (Ramanauskiene *et al.* 2016).

There are two subspecies of *M. officinalis* cultivated in Europe, the subsp. *altissima* (Sibthr. et Smith) Arcang., known as crete balm and the subsp. *officinalis* L. known as lemon balm.

Crete balm has been investigated in the past few years. The aroma and the composition of the essential oil show characteristic differences when compared to lemon balm. According to Božović *et al.* (2018) the essential oil contains mainly sesquiterpenes (caryophyllene-oxide, *cis*-caryophyllene and germacrene D) but only in traces, citral and citronellal.

The cultivation of lemon balm 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). Crete balm can be found in the Mediterranean region (Miceli *et al.* 2006). According to the literature, several pathogens affect lemon balm e.g. *Septoria melissae* Desm., *Oidium* spp., *Alternaria* spp. and *Puccinia menthae* Pers. *Septoria melissae* is responsible for the greatest damage to the plants (Nagy and Horváth 2010; Jadczak and Pizoń 2017; Wielgusz and Seidler-Łożykowska 2017). The pathogen may affect crete balm as well (Vanev *et al.* 1997). First symptoms usually appear on the older leaves in May in central Europe (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). On lemon balm, the disease very often leads to serious yield losses due to severe leaf fall. However, even a lower level of infection may influence the quality of the drug by decreasing the yield of the essential oil and altering its composition (D'Aulerio *et al.* 1995; Kowalska *et al.* 2014). It is notable that there are no available studies about the effect of *Septoria* infection on the yield or on the essential oil composition of crete balm.

Plant protection of lemon balm is often difficult in practice. The possibility of disease management by chemicals is restricted, due to strict regulation of the maximum allowed residue levels in herbal products (Kowalska *et al.* 2014; Bernáth and Zámbori-Németh 2015). There are only a few fungicides authorized for the protection of lemon balm in Hungary (Ocskó *et al.* 2019). Consequently, the sustainable and effective protection of lemon balm against phytopathogenic fungi seems to be quite difficult. Cultivation of tolerant or resistant genotypes is one of the most advantageous alternative methods of crop protection compared to the use of chemicals (Lynch *et al.* 2017). However, there are only a few references about the intraspecific variability of *M. officinalis* concerning resistance against pathogens including *S. melissae*. Meyers (2007) reports a moderate resistance of the cultivar 'Citronella' against powdery mildew disease. The cultivar 'Lemona' proved to be the least susceptible to the disease development and severity of the pathogen *S. melissae* (Kovács *et al.* 2019).

The aim of this study was to evaluate the susceptibility of three *M. officinalis* accessions including subsp. *altissima* to Septoria leaf spot under field conditions.

Materials and Methods

Plant material

The study was carried out in 2017 and 2018 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 had sandy soil with a slightly acidic (6.21) pH value, and high phosphorus (1,330 mg · kg^{-1}), moderate potassium (463 mg · kg^{-1}) and low nitrogen (36 mg · kg^{-1}) content. The humus content was 0.76%.

Weather conditions were similar in the two growing seasons. Distribution of rainfall had a similar tendency in both years. The sum of precipitation was 236 and 253 mm from the beginning of May to the end of August in the two years, respectively (Table 1). Plots were irrigated with 10–15 mm of water three times per week during arid periods in both years. Mean temperatures were 21.6 and 20.7°C in the investigated periods, respectively.

The following lemon balm accessions were involved in the experiment: *M. officinalis* subsp. *officinalis* 'Lorelei' a German registered variety (further on referred to as 'Lorelei') a cultivated form of the former subspecies, originated from field production near Wrocław, Poland (further on referred to as 'Wrocław') and a crete balm subspecies *M. officinalis* subsp. *altissima*, of an unknown origin (further on referred to as 'Altissima') were investigated. The seed material of 'Lorelei' and 'Altissima' were purchased from a commercial company (Jelitto Staudensamen GmbH). The accession 'Wrocław' was retrieved from the gene bank of the Department of Medicinal and Aromatic

Table 1. Sum of precipitation and mean temperatures of the experimental site in both vegetation periods (2017 and 2018) from April to August

2017													
	April	May			June			July			August		
Ten day period	III	I	II	III	I	II	III	I	II	III	I	II	III
Precipitation [mm]	11.20	35.60	6.20	3.60	0.40	3.40	30.00	44.60	6.20	42.80	34.40	27.60	1.00
Temperature [°C]	10.78	13.47	16.93	18.39	20.42	20.17	23.17	22.17	20.62	21.50	25.33	21.83	18.83
2018													
	April	May			June			July			August		
Ten day period	III	I	II	III	I	II	III	I	II	III	I	II	III
Precipitation [mm]	16.20	0.60	37.00	33.60	25.20	26.80	39.50	16.00	3.80	43.00	17.50	4.00	6.00
Temperature [°C]	17.36	19.64	16.82	20.61	22.05	21.78	18.59	20.00	21.56	23.61	24.24	22.80	19.90

Plants, Szent István University. Seedlings were grown in a greenhouse and 2 months old plants were planted outside on June 7th, 2017. The small plot (0.9 m × 2 m) trial was carried out in four replicates (5 plants · plot^{-1}) in both years. Plant spacing was 0.9 m × 0.4 m. After transplantation, the plants received 2 mm water three times per week for a fortnight to enhance rooting. Weed control was carried out mechanically. Harvesting of the plants in the first year was carried out once, on August 31st. In the second year, plants were harvested two times, on June 11th and on August 29th.

Identification of the pathogen

The experiment was performed under conditions of natural infection. Diseased plant debris was left in the field after the first year, which may have contributed to a more intense disease development in 2018.

Identification was carried out by symptomological observations followed by an examination of the morphological features of fungal structures with a Nikon Eclipse 50i cytosol microscope. The published descriptions (e.g. Nagy and Horváth 2010; Hoppe 2013) are in accordance with the symptoms observed on leaves and with the characteristics of investigated fruiting structures (Fig. 1).

Fig. 1. Symptoms of *Septoria melissae* Desm. on the leaves of *Melissa officinalis* subsp. *officinalis*

Disease assessment

Evaluation was carried out by the method described in our previous study (Kovács *et al.* 2019). Assessments of disease level were carried out weekly from the beginning of June to the end of August in 2017 and from the end of April to the end of August in 2018. In both years, all fully developed leaves of 2–4 fully developed shoots were assessed on each plant (altogether about 25–45 leaves · plant^{-1}).

The infection rate was defined for each plant (20 plants · accession^{-1}) by the following parameters: The disease incidence (DI) was calculated by the ratio of the infected leaves and the totally assessed ones in each plant and expressed in percentage. The disease severity index (DSI) was calculated by the formula of Towsend and Heuberger (Gartner 1971) based on the area of the necrotized tissue compared to the total surface of the leaf. Leaves were classified into the following five infection categories: 0 – healthy 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

Statistical analyses were carried out with the data of the selected assessments of both vegetation periods assuring the most representative results. Data were analysed by the IBM SPSS Statistics 25 software. Multivariate ANOVA (MANOVA) was used to define the difference between the investigated accessions considering their dependent values DI and the DSI.

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

All statistical analyses were carried out at 95% significance level.

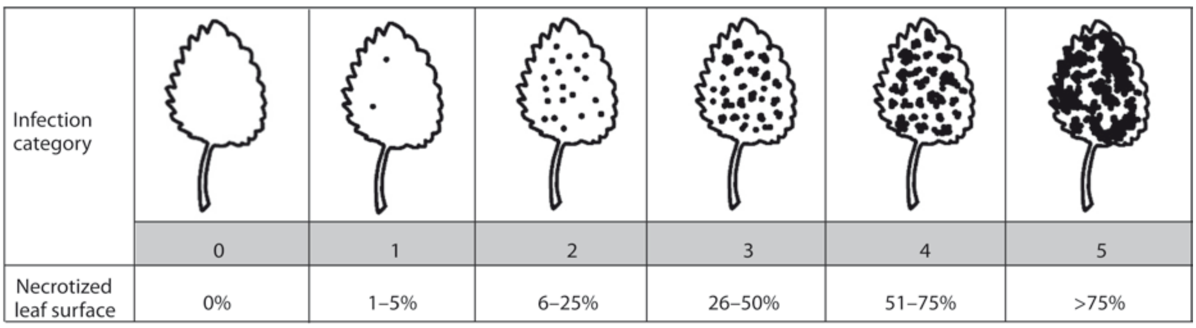

Fig. 2. Infection categories based on leaf symptoms

Results

The first symptoms appeared on the naturally infected leaves as early as in the second week after planting. Infection began to increase to higher levels at the beginning of August in the first vegetation period (Figs. 3–4). Concerning disease severity, differences between plant materials were remarkable from the end of July in the vegetation period of 2017. The highest ratio of the infected leaves was observed on cultivar 'Lorelei' at each assessment. The disease incidence and disease severity on the plots of cultivar 'Lorelei' were at least 10 times higher than the other accessions on August 16th. Before harvest in 2017, disease incidence and disease severity index reached 59.6% and 25.0, respectively. However, on the plots of the accessions of 'Altissima' and 'Wrocław', disease incidences were significantly lower by 38.9–68.1% and disease severity indexes by 15.4–20.8, respectively. According to statistical analysis the values of disease incidence and disease severity index were significantly higher on the plots of 'Lorelei' than on the other plant materials at the two selected assessments in 2017 (Fig. 5).

In 2018, assessments were carried out on the same plants. First leaf spots appeared in April, two weeks after the beginning of shoot development. Disease evaluation started at the beginning of May. As in the first year, the highest disease levels were observed on the plots of cultivar 'Lorelei' during the vegetation period of 2018 (Figs. 6–7). The lowest ratio of infected leaves was observed on the plants of 'Altissima' with an average of 28.1%. Before the first harvest, disease incidence reached 84.5% on the plots of 'Lorelei', however on the plots of other accessions the ratio of the infected leaves remained significantly lower by 34.0–50.4%

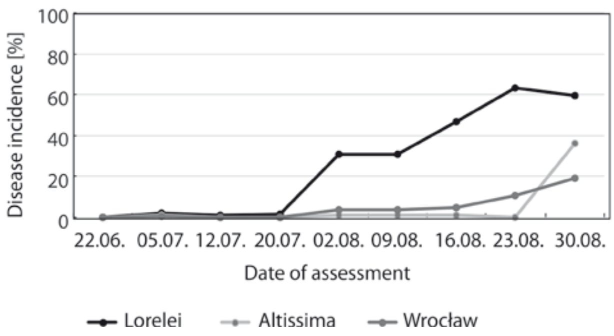

Fig. 3. Disease incidence (DI) on the three plant materials during the vegetation period of 2017

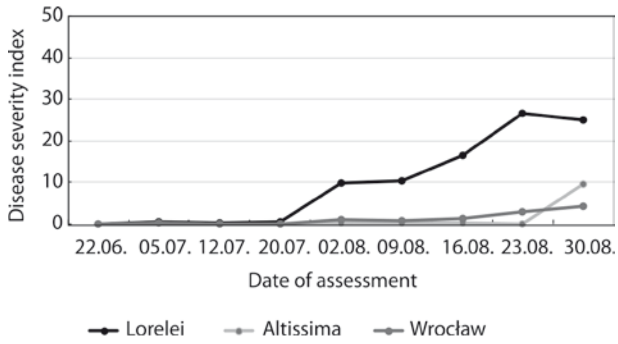

Fig. 4. Disease severity index (DSI) on the three plant materials during the vegetation period of 2017

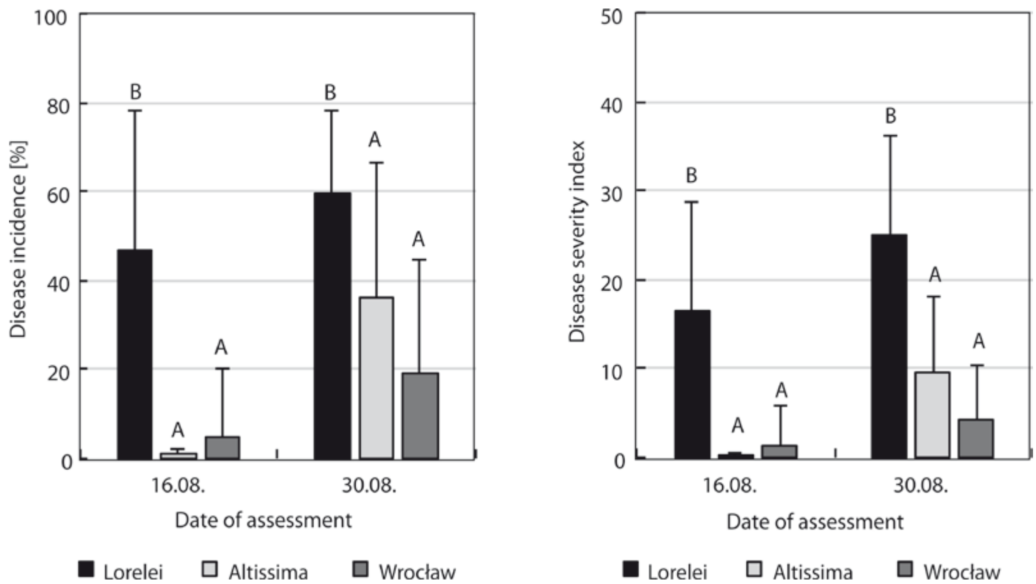

Fig. 5. Results of disease incidence (DI) and disease severity index (DSI) at the selected two assessments in 2017
Legend: letters refer to significant differences based on the Tukey (DSI 30.08.) and the Games-Howell (DI 16.08. and 30.08., DSI 16.08.) *post hoc* tests among the cultivars at each assessment separately

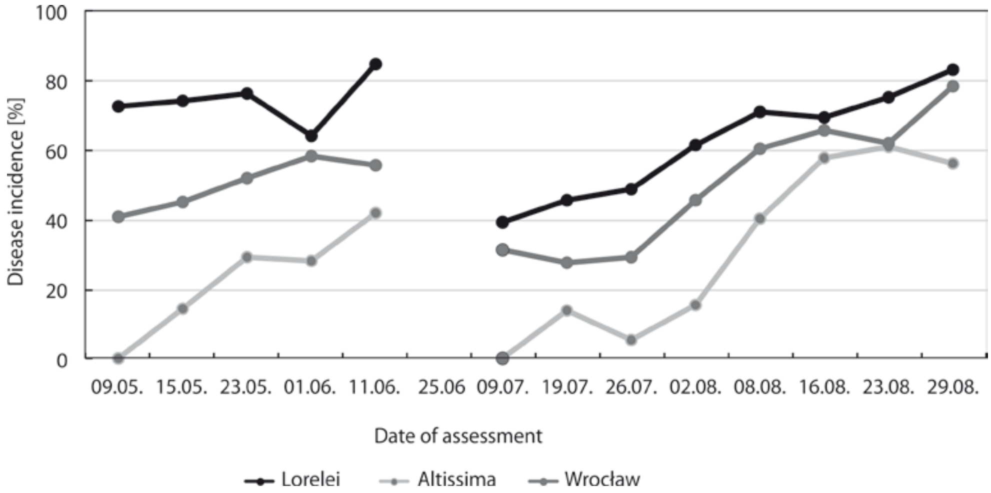

Fig. 6. Disease incidence (DI) on the three plant materials during the vegetation period of 2018

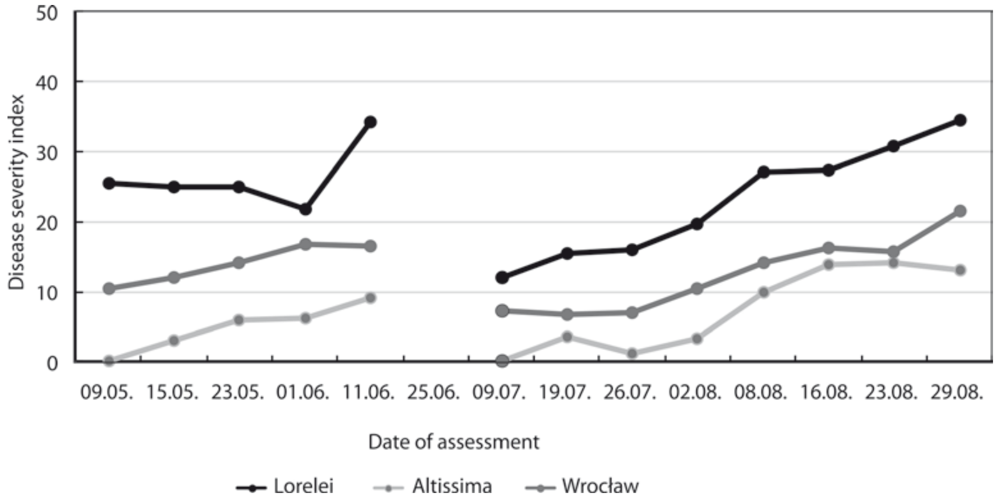

Fig. 7. Disease severity index (DSI) on the three plant materials during the vegetation period of 2018

(Fig. 8). The development of symptoms was the most intensive on the plants of 'Lorelei' as well. The calculated disease severity index was also high (34.3) in these plots. A similar trend in disease development was observed in the second part of the vegetation period of 2018. The disease incidence and disease severity index (58.8% and 17.7, respectively) remained the lowest on the plants of accession 'Altissima' by the end of summer (Fig. 9).

There was a higher ratio of infected leaves in the plots of 'Lorelei' and 'Wrocław' (82.9% and 78.2%, respectively) than in 'Altissima' plants. The disease severity indexes of all accessions were significantly different from each other.

The evaluated two years showed a similar tendency in the disease development of the pathogen on the different plant material. Lower disease incidence occurred on each accession in the first year, than in the second one. In both vegetation periods, accession 'Altissima' was the least susceptible to the infection. The variety 'Lorelei' showed the highest susceptibility. According to the results of the assessments, the susceptibility of both 'Wrocław' and 'Altissima' to the pathogen was similar in 2017. However, in 2018, 'Wrocław' showed an intermediate susceptibility (except DI on August 29th) between 'Lorelei' and 'Altissima'.

The fallen infected leaves which were left in the plots after the first year, served as an inoculum source and could have contributed to higher infection levels in the second year. The small difference in the sum of precipitation in the two years investigated, presumably did not influence the disease development, due to supplementary irrigation in the arid periods.

In light of our results, the selection of less susceptible cultivars may contribute to an effective and environmentally friendly plant protection method for the cultivation of lemon balm. However, further studies are needed to determine the exact physiological, biochemical and morphological background of the variable susceptibility of the intraspecific accessions and available cultivars.

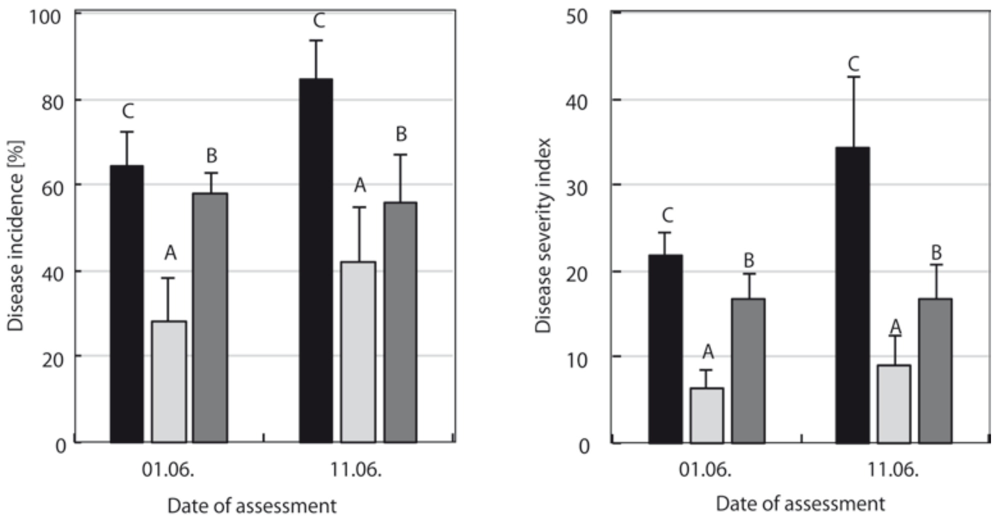

Fig. 8. Results of disease incidence (DI) and disease severity index (DSI) at the selected two assessments before the first harvest in 2018. Legend: letters refer to significant differences based on the Tukey (DI 11.06. and DSI 01.06.) and the Games-Howell (DI 01.06. and DSI 11.06.) *post hoc* tests among the cultivars at each assessment separately

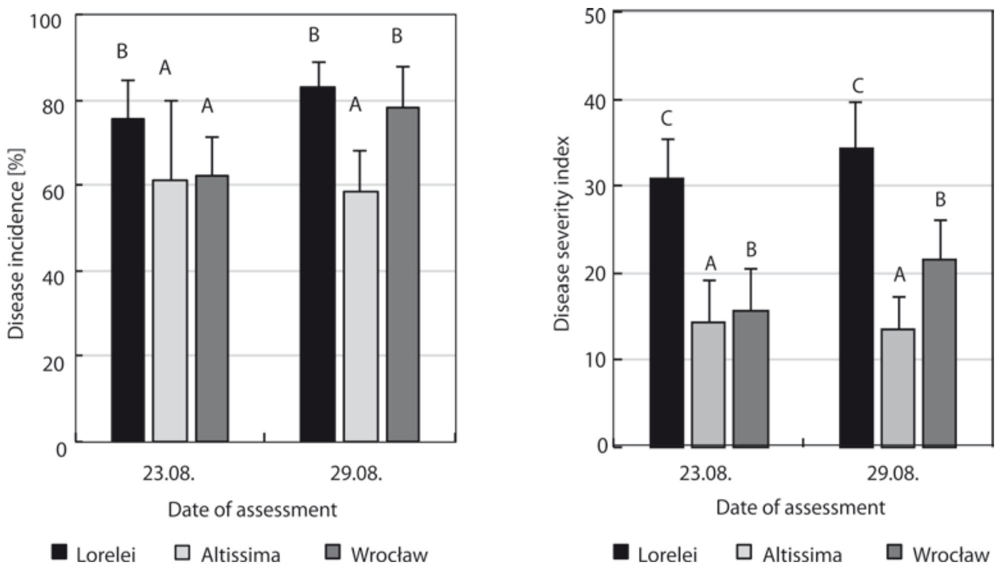

Fig. 9. Results of disease incidence (DI) and disease severity index (DSI) at the selected two assessments before the second harvest in 2018. Legend: letters refer to significant differences based on the Tukey (DI 29.08., DSI 23.08. and 29.08.) and the Games-Howell (DI 23.08.) *post hoc* tests among the cultivars at each assessment separately

Discussion

There seems to be a considerable intraspecific variability of lemon balm concerning the susceptibility to *S. melissae*. *Melissa officialis* subsp. *altissima* showed the lowest disease levels on almost all the assessments while the fastest symptom development and the highest infection levels were detected on cultivar 'Lorelei'. The susceptibility of the cultivated form 'Wrocław' was different in the two investigated years. In 2017, under low to medium disease pressure 'Wrocław' showed low susceptibility similar to subsp. *altissima*. However, in higher disease situation in 2018, 'Wrocław' became more susceptible than subsp. *altissima*. The presence of the fallen infected leaves may have served as an inoculum source and thus contributed to a higher infection level on the plantations. In accordance with our previous studies (Kovács *et al.* 2019), the breeding of disease tolerant *M. officinalis* varieties would be an effective tool in the plant protection of lemon balm. In order to develop the sustainable cultivation practice of this herb, further studies, including other varieties and cultivated forms of lemon balm, are needed. Further studies should also focus on environmental and agrotechnical factors influencing the infection level and spread of the disease on plantations.

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