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Leaf spot disease on lindens caused by the fungi *Cercospora microsora* Sacc. and *Gloeosporium tiliae* Oudem.

Helena Ivanová[1], Slávka Bernadovičová[2]

Department of Woody Plants Protection, Branch of Woody Plants Biology, Institute of Forest Ecology of the Slovak Academy of Sciences, Akademická 2, 949 01 Nitra, Slovak Republic,
[1]E-mail: nrueivan@savba.sk, [2]E-mail:nruebern@savba.sk

Abstract

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Certain of the growth characteristics of *Cercospora microsora* Sacc. and *Gloeosporium tiliae* Oudem. – causal agents of leaf spot diseases on lindens (*Tilia cordata* Mill.) in urban plantings in Slovakia were studied under laboratory conditions. Myceliar growth of *C. microsora* and *G. tiliae* was observed in pure hyphal cultures in relation to the medium and locality. In *Cercospora* study, one-way ANOVA has generally confirmed a statistically significant influence of both factors, medium and locality on growth rate of *C. microsora*, but in the case of the locality Nitra, the significant influence of the used media has not proved ($p > 0.05$). PDAg was generally shown as a medium inducing the most intensive growth in both localities (43.04 mm/4 days on average). Comparing the two localities, growth rate values from the locality Bratislava indicate unsuitability of water agar as a medium for the fast growth in culture. In *Gloeosporium* study, one-way ANOVA confirmed a significant influence of the factor medium as well as the locality on growth rate of *G. tiliae*. Influence of the used media was proven more markedly. Malt agar induced the most intensive growth in both localities (46.05 mm/4 days on average). Comparing the two localities, the samples from the locality Nitra showed in average the highest values of the growth rate. A Tukey test (ANOVA) separately conducted for the factors medium and the locality for both investigated fungal species, revealed the significant combinations of means ($p \leq 0.05$).

Key words

Cercospora microsora, Gloeosporium tiliae, growth rate, leaf spot, *Tilia cordata*

Introduction

Leaf spots are the most common diseases of shade and ornamental trees. Most of these diseases are promoted by cool weather, light and frequent rains, fog or heavy dews, high humidity, and crowded or shady plantings (Heimann and Mahr, 1997; Pataky, 1998). Many leaf spot diseases are caused by fungi which attack one or several tree species. The spreading of fungus basically depends on meteorological conditions, for example humidity, active solar radiation and temperature (Stakvilevičienė, 1999). Leaf spot is a dead spot on the leaf that is well distinct from the healthy tissue. These spots usually become conspicuous from late June through August. Leaf spot infections that start early in the growing season can lead to premature defoliation. Leaf spots commonly increase in number and size in late summer and early autumn as the leaves become senescent. If it occurs over two or more successive years, it can seriously weaken the tree, reduce its growth, and increase its susceptibility to bark borers, winter injury and other diseases (Pataky, 1998).

Species of the genus *Tilia* characterized by heart-shaped leaves, magnificent shady crowns and small golden flowers, known for their fragrance, prefer to grow on moist, fertile soils that are well drained and sufficiently aerated. Lindens that have been planted on harsh urban sites tend to lose their vigour and die prematurely. Sites that are not suitable for lindens include high situated areas exposed to wind and sun. Here, the trees are prone to diseases caused by fungi or by pests.

Activation of diseases of peripheral importance up to the present arises as a result of impact of climatic changes on woody plant species growing in Central Europe. Changed ecological conditions are reflected in decline in health state of greenery in urban environment. Temperature increase, longer growing season, high soil and air humidity in connection with escalated predisposition of woody plants participate substantially in activation of causal agents of numerous leaf diseases, where, besides powdery mildews and rusts, various leaf spots play important role (Jankovský, 2002). Since 2002, markedly increasing expansion of many fungal species participating in formation of leaf spots has been noticed. Exceptionally noticeable damage by the genus *Apiognomonia* to linden leaves after their sprouting was observed by Jankovský (2005), on beeches and plane trees by Kapitola et al. (2002).

On lindens, a leaf spot caused by the fungus *Cercospora microsora* Sacc. (teleomorph *Mycosphaerella millegrana* (Cook.) Schröet., *Mycosphaerella microsora* Syd.) causes circular brown spots with dark borders. When the spots are very numerous, the entire leaf may turn brown and fall off from the tree (Broembsen, 2005). Leaf spots on linden caused by *Gloeosporium tiliae* Oudem., syn. *Discula* sp. (teleomorph *Apiognomonia tiliae* (Rehm.) Höhn.) cause elongated light brown spots with a size of 1–20 x 1–15 mm situated close to the veins. The spots are more frequent near the tip, and they are bordered by a distinct black band. Leaf blight causes leaves to brown and fall. This causal agent of the leaf spot disease has become, up to the present time, one of the most widespread fungi on lindens in Slovakia.

The aim of the present study is to specify growth characteristics of *Cercospora microsora* (teleomorph *Mycosphaerella millegrana*) and *Gloeosporium tiliae* (teleomorph *Apiognomonia tiliae*) isolated on *Tilia cordata* growing in urban greenery, and on the basis of laboratory experiments to extend knowledge on *Cercospora* and *Gloeosporium* fungi in ecological conditions of Slovakia.

Material and methods

Material, isolation and cultivation

To determine grow rates of *Cercospora microsora* and *Gloeosporium tiliae* on *Tilia cordata*, leaf samples with characteristic spots were taken during growing season (from July to September 2005) from affected host trees growing in urban environment (street plantings) at two selected localities (Nitra, Bratislava). Leaf samples were collected from the lower parts of tree crowns. Altogether 50 samples were used for isolation from one location. Age of the evaluated trees varied from 20 to 60 years.

Samples were surface-sterilized in 70% ethanol and 15–20 minutes in sodium hypochlorite (1% available chlorine), rinsed in sterile distilled water (2–3 times) and dried carefully between filter papers. After surface sterilization, tissue samples were cut in small pieces (2–3 mm) and placed on 1% malt extract agar (MA) (10 g l^{-1} Difco agar, 7 g l^{-1}malt extract) and subsequently incubated in Petri dishes at 24–25 ºC, in the dark. Pure fungal cultures (from 15–25) were obtained after multiple purification from each investigated locality and one isolate from one location as a model sample was used in experiments.

Growth rates of *Cercospora microspora* and *Gloeosporium tiliae*

Myceliar growth was assessed on 10-day-old pure cultures grown in Petri dishes at 24–25 °C, in darkness. Three artificial media, 3% malt agar (MA) (30 g l^{-1} Difco agar, 21 g l^{-1} malt extract), 3% potato-dextrose agar (Difco) (PDAg) enriched with bromcresol green (50 mg l^{-1}) and 3% water agar (A) (30 g l^{-1} agar) were used in cultivation. The pH values of the cultivation media were adjusted to 6 with KOH. Agar column about 0.5 x 0.5 mm of parent mycelium was used for inoculation of plates.

Growth rates of pure cultures were obtained by recording of daily growths of mycelium (mm/day; with precision of 0.5 mm) during four days. Altogether 10 repetitions for each tested fungal species were made for each locality and medium, and five dishes with fungal colonies were examined in each replication.

Statistical analysis

The one-way analysis of variance (ANOVA) was used to assess the influence of two factors – locality and medium, on growth rate of *C. microsora* and *G. tiliae*. Overall growth rate after four days was considered (differences in individual time intervals were not compared). Influence of the used medium was tested separately for each locality, as well as influence of the locality for each medium.

A Tukey test (ANOVA) was performed to identify significantly different combinations of growth rate means.

The statistical package STATISTICA-7 (StatSoft) was used for all analyses.

Results and discussion

According to our recent observations, the fungi *Apiognomonia tiliae* (Rehm.) Höhn. and *Mycosphaerella millegrana* (Cook.) Schröet belong to the most frequent causal agents of leaf spot diseases on lindens. Former

results of many studies in Slovakia (Juhásová, 1975, 2002) as well as other countries (Laubert, 1904; Byther and Davidson, 1979; Sinclair and Johnson, 1997; Heimann and Mahr, 1997; Pataky, 1998; Stakvilevičienė, 1999; Stipes, 2000; Kapitola et al, 2002; Szabó, 2003; Jankovský, 2002, 2005; Mielke and Daughtrey, 2005) have confirmed its incidence, although in low intensity and extent.

Specific symptoms of leaf spot – anthracnose somewhat vary, depending on the tree species infected. Anthracnose on linden caused by *Apiognomonia tiliae* may cause defoliation of linden trees. Damage of this type usually occurs after unusually cool, wet weather during bud break. According to Stipes (2000) and Jankovský (2002, 2005), single attacks are seldom harmful to the tree, but yearly repeated infections will cause reduced growth and may predispose the tree to other stresses. Early loss of leaves repeated over several successive years weakens the tree and predisposes it to attack by borers and to winter injury (Ragazzi et al., 2002). Premature leaf fall reduces the shade and ornamental value of the tree (Mielke and Langdon, 1986; Daughtrey et al., 1988; Stipes, 2000; Juhásová, 2002).

According to Laubert (1904) and Juhásová (2002), bullously hollowed acervuli of anamorph stage (*Gloeosporium tiliae*) onto upper and lower leaf side contain oval, nonseptate hyalin conidia produced in fruiting bodies on infected parts of new leaves. Conidia, produced in large numbers, are also spread from leaf to leaf by wind and splashed by rain. The rapid arising and spreading of anthracnose in summer and autumn occurs by means of these spores (Stipes and Campana, 1981; Broembsen, 2005).

Pataky (1998) has described disease on linden trees caused by species of *Cercospora* as a formation of circular to angular, small to large, gray or brown spots with dark margins. Some spots may drop out and turn to strip-bordered shot-holes. The spots may be numerous and cause the leaf to turn brown and fall prematurely. Donaubauer (1999) confirmed the occurrence of small necroses on lindens growing in west and middle Austria. The spots formed by *Cercospora microsora* appeared from late July through August. According to Juhásová (2002) and Broembsen (2005), leaf spot on linden is caused by the fungus *Cercospora microsora* Sacc. The spots are small, circular or circular to oval, brown with dark borders. When the spots are very numerous, the entire leaf may turn brown and fall off from the tree.

Although leaves and other plant tissues infected with the fungus usually persist from one growing season to the next, cool, rainy periods in early to mid-spring are often not long enough for the fungus to grow, multiply, and infect new leaves. Consequently, the presence and severity of leaf spot diseases are variable from year to year. Leaf spots are more likely to develop when there are extended periods of cool, moist weather in April, May, and June, when the growth of new leaves is progressive.

In Table 1 are summarized the basic characteristics and differences of fungi *Apiognomonia tiliae* and *Mycosphaerella millegrana*, the most frequent causal agents of leaf spot diseases on lindens.

The external factors as temperature, nutrition, humidity and pH value of the environment as well as conditions of growth *in vitro* (medium, temperature, pH) have an important influence on the pathogen growth (Juhásová, 1975). Stakvilevičienė (1999) presents the results of investigation on environmental influence on distribution of cercosporoid fungi in Lithuania in 1992–1998. According to this author, the spread of such a type of fungi basically depends on meteorological conditions. The distribution of brown leaf spot (*Cercospora microsora*, *Passalora microsora*) of trees is independent on presence of pollution, industry or the fact whether the site is situated in a large city or in a small town (health resort).

Kaneko and Kaneko (2004) observed only little differences among three artificial media (2% MA, PDA and LCA) regarding fungal growth rates, but growth rates differed among fungal species. *Mycosphaerella buna, Ascochyta fagi, Periconiella* sp. and *Tritirachium* sp. grew well between 15 and 30 °C. Insignificant or even no growth was observed at 5 or 35 °C by each fungus. Zimmermannová-Pastirčáková (2002) has summarised the effect of different pH values of medium on radial growth of *Phyllosticta sphaeropsoidea* colonies. Mycelium of this fungus grew on malt extract agar within the range of pH 3–12. Radial growth rate of isolates of this fungus was the highest over the optimum pH range (6–8). The pH values of the cultivation media used by our experiments were adjusted to a pH value of 6 with KOH. According to Sundari and Adholeya (2003), the substrate pH should not only determine the growth rate of the fungus but it also limits further proliferation of the fungus in the medium.

This study was focused on the specification of fungi biology on the basis of *in vitro* growth rate of *Cercospora microsora* and *Gloeosporium tiliae*. Average daily growths (mm/day) of *C. microsora* and *G. tiliae* isolated on *Tilia cordata* at two localities on three different media, after 24, 48, 72 and 96 hours of cultivation are summarised in Table 2.

The fungus *Cercospora microsora* in cultivation procedure has formed aerated light-pink, lately, due to cercosporin, purplecoloured colonies. In *Cercospora* study, using the one-way ANOVA, a significant influence of the two factors, culture medium ($F_{Bratislava} = 112.61$, $p = 0.00$; $F_{Nitra} = 1.42$, $p = 0.25$) and locality ($F_{MA} = 22.78$, $p = 0.00$; $F_{PDAg} = 71.06$, $p = 0.00$; $F_{A} = 45.41$, $p = 0.00$) on growth rate of investigated fungus was confirmed in general, although the effect of these factors

has not been proved unambiguously in all cases. In the case of the locality Nitra, the significant influence of the used media has not been proved ($p > 0.05$). Growth rates of *C. microsora* are shown in Fig. 1.

PDAg was generally showed as the most suitable medium, inducing the most intensive growth in both localities (43.04 mm/4 days on average), although the significant difference was not proved in every case. On the contrary, samples from these localities showed the lowest growth on the medium A (32.53 mm/4 days on average). The MA induced 39.35 mm/4 days, on average.

Comparing the two localities, the effect of this factor is not such unambiguous. The samples from the locality Bratislava showed on average the highest values of the growth rate in the cases of PDAg (49.8 mm/4 days) and MA (43.05 mm/4 days). Only in the case of medium A, it was a lower value. The samples from the locality Nitra grew evenly without regard to the medium (35.21 mm/4 days on average). Samples from this locality growing on two media (PDAg, MA) generally showed the slowest growth. Hovewer, in the case of medium A, samples from Bratislava proved slower growth than isolates from Nitra. Thus, growth rate values from the locality Bratislava indicate unsuitability of medium A for the fast radial growth. When using PDAg and MA, the isolates grew faster than samples from Nitra.

A Tukey test (ANOVA) separately conducted for the factors medium and the locality revealed the significant combinations ($p \leq 0.05$) of means (in bold in Tables 3, 4). The test has revealed the significant differences between average growth rate values of the samples from Bratislava cultivated on PDAg, MA and A. On the contrary, in the case of isolates from the locality Nitra, the differences between three media were statistically insignificant.

Table 1. Comparison of basic characteristics of *Apiognomonia tiliae* and *Mycosphaerella millegrana* on linden trees (*Tilia* sp.) (Pataky, 1998, modified)

Apiognomonia tiliae,* *anamorph Gloeosporium tiliae	***Mycosphaerella millegrana,* *anamorph Cercospora microsora***
General symptoms	
1–20 x 1–15 mm areas	3–4 mm dead areas
light brown with black margins	at first darkgreen, later yellow, tan or brown
small to large, circular to elongated	small, circular to oval
Occurrence	
anywhere on a leaf, most often near the tip, especially along main leaf veins	on the upper and lower side of leaves
Early symptoms	
appearing in May before complete maturity of leaves; round, brown, irregular, dark-bordered spots with varying dimensions	appearing from late July through August
Spores	
oval, nonseptate, hyalin	filamentous, septate (3–8), light brown
10–13 x 4–5 μm	35–100 μm
during rainy periods in spring	cool, moist weather from April to June
Fruiting body	
in dead tissues	in dead tissues of many older spots
Spread	
by wind, by splashing rains	by air currents, by splashing rain, by insects
Primary infection	
by ascospores which are discharged from leaves	by ascospores
Germination	
in presence of moist conditions	in presence of free water
Causes	
brown and fallen leaves, defoliation	leaf turned brown and falling prematurely

Table 2. Average daily growth (mm/day) of *Cercospora microsora* and *Gloeosporium tiliae* isolated on *Tilia cordata* at two localities on three different media, after 24, 48, 72 and 96 hours of cultivation

Cercospora microsora

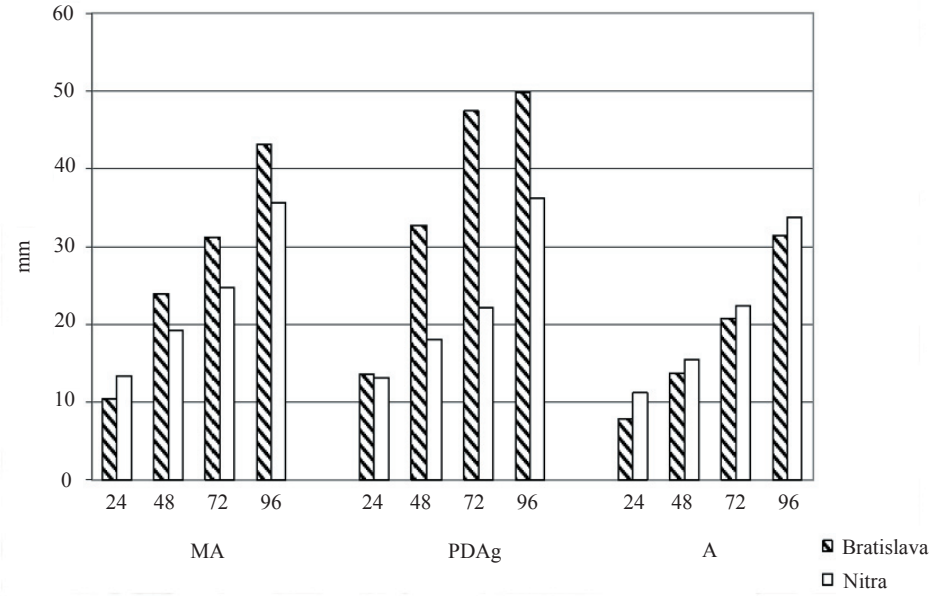
No.	Locality Bratislava												Locality Nitra											
	MA				PDAg				A				MA				PDAg				A			
	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
1	10.5	23.0	35.0	42.5	11.5	32.0	44.5	47.0	8.5	12.0	18.5	31.5	14.0	20.5	25.5	36.0	13.0	17.0	22.5	34.0	10.5	14.0	23.0	36.5
2	10.0	21.0	31.5	42.0	12.5	33.0	46.0	46.5	8.0	11.5	17.5	27.5	14.0	21.5	25.5	36.5	15.5	19.5	22.5	33.5	11.0	17.0	23.5	37.5
3	9.5	23.0	32.0	42.0	12.5	31.5	46.5	46.0	8.0	14.0	23.5	31.5	12.0	18.0	23.0	32.0	14.5	20.5	28.0	40.0	12.5	17.0	23.5	40.0
4	10.5	23.5	33.0	44.0	14.0	32.5	47.5	50.0	9.0	12.0	23.0	34.5	12.5	19.0	23.5	35.5	13.0	17.5	21.5	38.5	11.5	17.0	27.0	43.5
5	11.0	26.5	36.5	43.5	12.5	33.5	48.5	50.0	8.0	12.5	22.5	33.5	12.0	22.0	26.0	33.5	13.0	19.5	24.0	40.5	11.5	19.0	23.0	34.0
6	9.5	20.0	31.5	40.5	13.0	34.5	48.0	51.0	7.5	12.0	21.5	33.0	15.0	20.0	25.5	36.0	14.0	17.5	22.5	39.5	11.0	18.5	23.0	33.5
7	10.0	25.0	35.5	44.5	11.5	32.0	44.5	51.5	7.5	12.0	24.0	33.5	14.0	19.0	24.5	38.5	12.5	17.5	21.5	34.5	10.0	17.0	20.0	28.5
8	10.5	25.5	37.0	43.0	15.0	29.0	47.0	52.5	7.5	14.0	19.5	31.0	12.5	17.5	25.5	35.5	13.5	18.5	20.5	39.0	11.5	17.0	19.5	27.5
9	11.0	26.0	37.5	44.0	14.0	36.0	52.5	51.5	7.0	12.0	20.5	34.0	13.5	15.0	24.5	36.0	11.5	17.0	19.5	32.0	10.5	17.5	20.0	26.0
10	11.0	26.5	37.0	44.5	14.0	33.0	49.0	52.0	8.0	13.5	18.0	23.5	13.0	19.0	24.0	37.0	10.5	15.0	19.0	31.0	12.0	18.5	21.5	30.0
Average	**10.4**	**24.0**	**31.1**	**43.1**	**13.1**	**32.7**	**47.4**	**49.8**	**7.9**	**13.8**	**20.9**	**31.4**	**13.3**	**19.2**	**24.8**	**35.7**	**13.1**	**18.0**	**22.2**	**36.3**	**11.2**	**15.6**	**22.4**	**33.7**

Gloeosporium tiliae

No.	Locality Bratislava												Locality Nitra											
	MA				PDAg				A				MA				PDAg				A			
	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
1	12.5	23.0	32.0	43.0	10.0	21.0	29.0	34.5	11.0	17.0	27.5	37.0	11.5	18.5	38.5	54.5	8.5	14.0	24.0	37.5	9.0	15.5	25.5	36.5
2	11.0	21.0	34.0	41.5	11.0	20.0	25.5	35.0	9.5	16.5	25.0	35.0	12.0	24.0	37.5	52.5	7.5	19.0	28.0	43.0	10.0	19.5	31.5	37.5
3	10.5	22.5	32.0	41.5	9.5	15.5	23.0	30.0	11.0	16.5	25.5	35.0	12.0	26.0	39.5	52.0	7.5	18.0	27.5	38.5	10.5	20.0	30.5	41.5
4	10.5	21.0	31.5	40.0	10.5	17.5	24.5	35.0	10.5	17.5	26.0	35.5	11.5	23.0	33.0	47.5	9.0	20.0	29.0	39.5	11.0	22.5	33.5	45.5
5	9.0	22.5	32.0	39.5	11.0	20.5	24.5	34.0	10.5	16.5	25.0	34.5	12.0	23.5	35.0	49.5	7.5	19.5	28.0	39.0	8.0	19.0	30.0	38.5
6	13.0	25.5	34.5	46.5	10.5	19.5	24.5	34.0	11.5	17.5	27.0	39.5	10.5	20.0	31.0	46.0	8.5	18.5	26.5	39.5	10.5	20.0	32.0	44.0
7	10.0	19.0	30.0	40.5	10.5	20.5	29.0	37.0	10.0	15.5	23.0	34.0	11.5	22.5	32.0	48.5	8.0	18.5	27.0	38.5	9.5	23.0	35.5	46.5
8	11.5	23.5	32.0	43.5	9.5	17.5	24.5	30.0	9.5	15.0	23.5	34.0	9.5	21.5	31.0	47.5	9.0	18.0	25.5	39.5	8.5	22.5	32.0	39.0
9	12.0	21.0	31.0	40.5	10.5	19.0	25.5	33.5	9.5	14.5	25.0	37.5	12.0	25.5	39.5	53.5	10.0	16.0	24.5	38.5	10.0	23.5	38.0	48.0
10	11.0	19.5	25.0	40.0	9.5	19.5	24.5	32.5	9.0	14.0	23.5	35.5	11.5	26.5	38.0	53.0	8.0	15.5	23.5	37.5	11.0	18.5	34.5	45.0
Average	**11.0**	**21.9**	**31.4**	**41.7**	**10.3**	**19.1**	**25.5**	**33.6**	**10.2**	**16.1**	**25.1**	**35.8**	**11.4**	**23.1**	**35.5**	**50.5**	**8.4**	**17.7**	**26.4**	**39.1**	**9.8**	**20.4**	**32.3**	**42.2**

* MA – 3% malt agar, PDAg – 3% potato-dextrose agar enriched with bromcresol green, A – 3% water agar

In cultivation, the fungus *Gloeosporium tiliae* has formed aerated, woolly, white to light-yellow colonies. In *Gloeosporium* study, using one-way ANOVA, a significant influence of both factors, culture medium ($F_{Bratislava} = 41.96$, $p = 0.00$; $F_{Nitra} = 36.34$, $p = 0.00$) and locality ($F_{MA} = 31.36$, $p = 0.00$; $F_{PDAg} = 16.65$, $p = 0.00$; $F_{A} = 11.86$, $p = 0.00$) on growth rate of the investigated fungus was confirmed. Influence of the used media were proved more markedly. Growth rates of *G. tiliae* are shown in Fig. 2.



MA – 3% malt agar, PDAg – 3% potato-dextrose agar enriched with bromcresol green, A – 3% water agar
Fig. 1. Growth rates of fungus *Cercospora microsora* on different media and two localities, after 24, 48, 72 and 96 hours of cultivation

Table 3. Influence of medium on growth rate of *Cercospora microsora* and *Gloeosporium tiliae* isolated on *Tilia cordata* and combinations of growth rate means compared in a Tukey test. Significant combinations ($p \leq 0.05$) are in bold

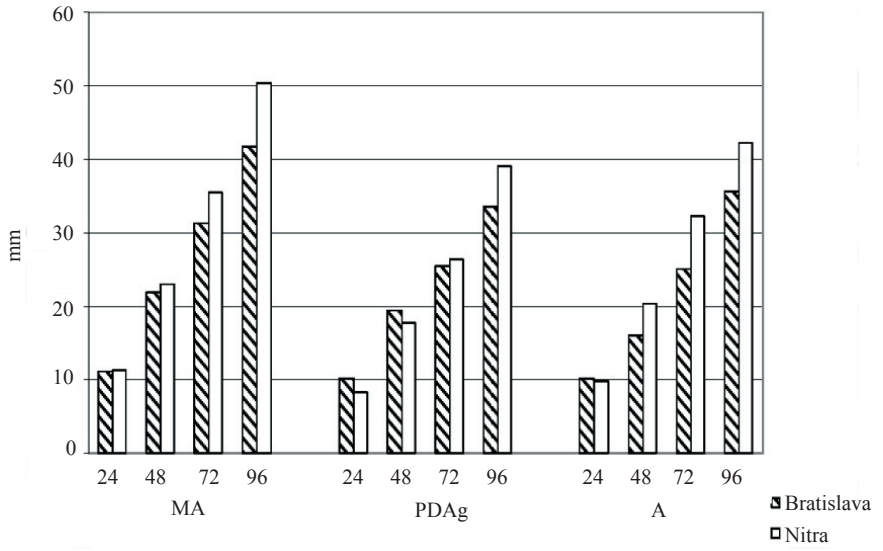
	Cercospora microsora					*Gloeosporium tiliae*			
				Locality Bratislava					
Medium	MA	PDAg	A	*Average*	***Medium***	MA	PDAg	A	*Average*
MA	–	**0.000121**	**0.000119**	43.05	MA	–	**0.000127**	**0.000131**	50.45
PDAg	**0.000121**	–	**0.000119**	49.80	PDAg	**0.000127**	–	0.080465	39.10
A	**0.000119**	0.000119	–	31.35	A	**0.000131**	0.080465	–	42.20
				Locality Nitra					
Medium	MA	PDAg	A	*Average*	***Medium***	MA	PDAg	A	*Average*
MA	–	0.918852	0.444100	35.65	MA	–	**0.000127**	**0.000128**	41.65
PDAg	0918852	–	0.247149	36.28	PDAg	**0.000127**	–	0.058571	33.55
A	0444100	0.247149	–	33.70	A	**0.000128**	0.058571	–	35.75

MA – 3% malt agar, PDAg – 3% potato-dextrose agar enriched with bromcresol green, A – 3% water agar

Table 4. Influence of locality on growth rate of *Cercospora microsora* and *Gloeosporium tiliae* isolated on *Tilia cordata* and combinations of growth rate means compared in a Tukey test. Significant combinations ($p \leq 0.05$) are in bold

Cercospora microsora				***Gloeosporium tiliae***			
			Medium MA				
Locality	Bratislava	Nitra	*Average*	***Locality***	Bratislava	Nitra	*Average*
Bratislava	–	**0.000119**	43.05	Bratislava	–	**0.000246**	33.55
Nitra	**0.000119**	–	35.65	Nitra	**0.000246**	–	39.10
			Medium PDAg				
Locality	Bratislava	Nitra	*Average*	***Locality***	Bratislava	Nitra	*Average*
Bratislava	–	**0.000119**	49.80	Bratislava	–	**0.000159**	41.65
Nitra	**0.000119**	–	36.28	Nitra	**0.000159**	–	50.45
			Medium A				
Locality	Bratislava	Nitra	*Average*	***Locality***	Bratislava	Nitra	*Average*
Bratislava	–	0.212677	31.35000	Bratislava	–	**0.000219**	35.75
Nitra	0.212677	–	33.70000	Nitra	**0.000219**	–	42.20

MA – 3% malt agar, PDAg – 3% potato-dextrose agar enriched with bromcresol green, A – 3% water agar



MA – 3% malt agar, PDAg – 3% potato-dextrose agar enriched with bromcresol green, A – 3% water agar
Fig. 2. Growth rates of fungus *Gloeosporium tiliae* on different media and two localities, after 24, 48, 72 and 96 hours

MA was the most suitable medium, inducing the most intensive growth in both localities (46.05 mm/4 days on average). On the contrary, samples showed the lowest growth on the PDAg (36.33 mm/4 days on average). The A induced 38.98 mm/4 days, on average.

Comparing the two localities, the effect of this factor is not such unambiguous. The samples from Nitra showed on average the highest values of the growth rate. Just in the case of PDAg, it was slightly lower.

The Tukey test separately conducted for the factors medium and locality revealed the significant combinations ($p \leq 0.05$) of means (in bold in Tables 3, 4).

According to Mielke and Daughtrey (2005), in case of a similar anthracnose fungus, *Discula* sp.

causing dogwood anthracnose, the fungus grows slowly on either malt or potato agar. Colonies are appressed, granular and white, and darken with age. Sporulation of *Discula* sp. in culture is highly variable and enhanced by amending media with dogwood tissue or extract. Juhásová et al. (2006) has studied the biology of anthracnose fungi *Gnomonia leptostyla* and *Marssonina juglandis* on *Juglans regia*. Statistically significant differences between three different media (2% water agar, 2% Czapek Dox agar, 2% maltose agar) and between the times of cultivation were identified by multiple range analysis. The level of variability between the localities was statistically insignificant.

The inoculum of these fungi overwinters in fallen leaves, so raking and removal of fallen leaves in autumn is needed. Commonly-used raking and liquidation of fallen leaves are recommended to reduce the chance of infection in the following season. It is also important to support the vigour of trees through adequate water supply during dry periods and through fertilization in the spring. It is necessary to reduce humidity around the tree by avoiding excessive watering in late afternoon or evening and by appropriate spacing of plants, ensuring them good air circulation.

Usually, anthracnose disease does not require chemical controls every year, but repeated severe infections may justify a spraying. Table 5 gives a list of protection measures used in control of two anthracnose diseases caused by *Gloeosporium tiliae* and *Cercospora microsora* on linden trees. Protective measures are generally preferred. If chemical control is desired, spraying with a fungicide containing mancozeb at bud-swelling, repeated again two times during leaf expansion (in most years, this should be at 10–14 day intervals) is effective. If fungicides are used, sprays must be applied on a preventative basis, before the infection takes place. Spraying large trees may be impractical and unnecessary, especially in dry springs. Sanitary measures are important for reducing the amount of fungal inoculum available for formation of new infections. Heimann and Mahr (1997) considered a similar protective control for *Cercospora* leaf spot diseases. According to these authors, the disease can be controlled by using fungicidal sprays containing Bordeaux. In this case, two or three applications are needed at 7- to 21-day intervals, usually as soon as the buds start to open and the leaves begin to expand, and long before the leaves are visibly infected. Spraying fungicides after the disease will evidently reduce secondary infections, but it will not eliminate infections that have already started. Additional sprays may be necessary after prolonged rainy periods. According to Nix (2005), the key to effective chemical control is a correct timing of protective fungicide sprays which is different for different fungi. Duoglas and Cowles (2005) assert, that *Cercospora* leaf spot is generally an aesthetic problem, consequently, fungicides are rarely needed. Since this disease is not usually a serious problem for the health of trees, chemical controls are not necessary in general. According to Pataky (1998), applications of spray with the aim to control diseases caused by *Cercospora* species are needed rarely, if ever.

Table 5. Comparison of control measures used by leaf spot diseases caused by fungi *Gloeosporium tiliae* and *Cercospora microsora* (Heimann & Worf, 1997, modified)

Gloeosporium tiliae	***Cercospora microsora***
Preventive control	
Rake up and remove infected leaves	Compost, burn
Leaves - shred, compost or burn	Reduce humidity levels around the tree by avoiding overhead watering in late afternoon or evening
Thinning – improve air movement	Spacing for good air circulation
– promote faster drying of the leaves	
Other management practices	
Fertilizing infected trees – month after the average date of the first frost or early spring abouth month before the date of the last frost to increase tree vigor	
Chemical control	
Spray with a fungicide containing mancozeb	Using fungicidal sprays containing Bordeaux mixture

According to our experiences, above mentioned protective control measures are not generally guaranteed for most leaf spots. Although the fallen leaves are often collected and then composted, burned or removed, there is a little evidence that these practices could significantly reduce infection in the following spring and summer. If severe leafspotting or defoliation occurs for several years, chemical control is probably necessary.

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Listové škvrnitosti líp spôsobené hubami *Cercospora microsora* Sacc. a *Gloeosporium tiliae* Oudem.

Súhrn

V súvislosti so zaznamenaním zvýšeného výskytu listových škvrnitostí líp v mestských výsadbách spôsobených hubami *Cercospora microsora* a *Gloeosporium tiliae* sme v laboratórnych podmienkach skúmali a následne štatisticky vyhodnotili rýchlosť rastu hýf mycélia uvedených húb. Čisté kultúry skúmaných húb sa hodnotili vo vzťahu k druhu použitého média (3 %-ný zemiakovo-dextrózový agar, 3 %-ný sladinový agar, 3 %-ný vodný agar) a k lokalite výskytu a odberu vzoriek (Bratislava, Nitra).

Jednofaktorovou ANOVA sme potvrdili štatisticky významný vplyv oboch faktorov, média a lokality na rýchlosť rastu huby *C. microsora*, hoci v prípade lokality Nitra sa signifikantný vplyv použitého média nepotvrdil ($p > 0,05$). Najintenzívnejší rast kultúr z oboch lokalít (v priemere 43,04 mm/4 dni) sa zaznamenal na zemiakovo-dextrózovom agare. Pri porovnávaní dvoch lokalít, hodnoty rýchlosti rastu hýf mycélia huby *C. microsora* na lokalite Bratislava poukázali na nevhodnosť použitia vodného agaru pre rast skúmanej huby.

Pri štúdiu huby *G. tiliae*, jednofaktorová ANOVA potvrdila signifikantný vplyv média a lokality na rýchlosť rastu skúmanej huby, pričom vplyv použitého média sa prejavil výraznejšie. Sladinový agar indukoval najintenzívnejší rast izolátov huby z oboch lokalít (v priemere 46,05 mm/4 dni). Porovnávaním lokalít, kultúry zo vzoriek z lokality Nitra dosahovali priemerne najvyššie hodnoty rýchlosti rastu. Tukeyov test (ANOVA) vykonaný samostatne pre faktory médium a lokalita pre oba druhy skúmaných húb ukázal signifikantné kombinácie priemerov hodnôt rastu hýf mycélia ($p \leq 0.05$).