Influence of Temperature and Species Origin on *Fusarium* spp. and *Microdochium nivale* Pathogenicity to Wheat Seedlings

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Abstract

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The influence of temperature and species origin on the *in vitro* growth rate and pathogenicity of *Fusarium* and *Microdochium nivale* (*F. avenaceum, F. culmorum, F. graminearum, F. poae*, and *M. nivale*) to wheat seedlings was examined. The mycelial growth of *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, and *F. poae* was the fastest at 25°C, and of *M. nivale* at 15°C. The isolates of *F. culmorum*, *F. graminearum* and *F. poae* originating from mountain regions grew significantly faster at 15°C than those from flatland regions. The isolates from flatland regions grew significantly faster at 25°C than those from mountain regions. *F. culmorum* and *F. graminearum* were the most pathogenic species to the root development. The retardation of wheat grain germination caused by the tested species was assessed in descending order: *F. culmorum*, *F. graminearum*, *M. nivale*, *F. avenaceum*, *F. poae*. The biomass growth retardation at 15°C was assessed in descending order: *F. culmorum*, *F. graminearum*, *M. nivale*, *F. avenaceum*, *F. poae*; at 25°C as follows: *F. graminearum*, *F. culmorum*, *F. avenaceum*, *M. nivale*, *F. poae*. The isolates of *M. nivale* and *F. poae* originating from mountain regions were significantly more pathogenic than those from flatland regions. The results suggest that there exist different temperature ecotypes and pathotypes of *Fusarium* species and *Microdochium nivale* across the territory of the Slovak Republic.

Keywords: *Fusarium*; wheat; seedlings; pathogenicity; *in vitro* growth; ecotypes

Fusarium diseases of wheat (seedling blight, foot rot, scab and *Fusarium* head blight – FHB) are caused by a number of species, mostly by *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* and *M. nivale* (Sutton 1982; Šrobárová & Eged 1992; Hudec & Roháčik 2003). *F. graminearum* predominates in North America and in Southern Europe, while *F. culmorum* and *M. nivale* predominate in cooler climates in Europe (Parry *et al.* 1995). Most cultivars of barley and wheat are susceptible to these pathogens and resistance to scab is race non-specific (Mesterházy *et al.* 1999). FHB in wheat results in quality and economic losses for the grain industry. Along with the reduced yield of kernels, economic losses arise from the accumulation of mycotoxins which can make infected kernels unfit for human and animal consumption and feeding. The milling and baking quality is also altered when grains are contaminated by *Fusarium* infection and mycotoxins (Wong *et al.* 1995).

Factors such as temperature, humidity and other environmental factors have a great importance for the incidence and severity of certain *Fusarium* species (Vigier *et al.* 1997). These factors are also important in mycotoxicosis epidemiology, because the production of mycotoxins by the different species is differentially affected by some environmental factors such as temperature (Di Menna *et al.* 1991; Jimenéza *et al.* 1996). Infested seed may be partly responsible for the *Fusarium* disease spreading. A

number of studies have examined the effects of FHB on seed germination and subsequent stand establishment (Gilbert & Tekauz 1995; Wong *et al.* 1995). Leaving *Fusarium*-damaged kernels on the soil at harvest can provide a primary source of inoculum for the development of seedling blight and other forms of wheat fusariosis (Parry *et al.* 1995).

Host range and climatic factors influence the growth, survival, spreading and hence the incidence of *Fusarium* species and the crop damage. The influence of origin and climatic factors on *Fusarium* diseases is complicated by the fact that *Fusarium* species are able to cause the diseases individually or in complex. There are some reports on how the *Fusarium* species differentially respond to different environmental variations, mostly temperature, isolate origin and humidity (Conrath *et al.* 2002).

The aim of this paper was the analysis of *Fusarium* spp. and *Microdochium nivale* isolates (originating from different locations of the Slovak Republic) focused on their *in vitro* growth, sensitivity to cultivation temperature, and their pathogenicity to wheat seedlings.

MATERIALS AND METHODS

Fusarium ***isolate collection***. Twenty isolates of *Fusarium culmorum*, *F. graminearum*, *F. avenaceum*, *F. poae* and *Microdochium nivale* were collected in different locations of Slovakia: Želiezovce, Sládkovičovo, Bučany, Nitra, Vígľaš-Pstruša, Malý Šariš (Figure 1). The chosen species are most frequently isolated from wheat spikes in Europe (Bottalico & Perrone 2002). The locations are characterised in Table 1. All strains were isolated from wheat seedlings. The monospore isolates were subcultured (incubated at 20°C under 12/12 photoperiods, 4000 Lux, none UV light) and stored on potato-dextrose agar (PDA).

Estimation of *in vitro* ***growth rate***. The temperature sensitivity was assessed by analysing the *in vitro* growth rate on PDA at 15°C and 25°C. The PDA plates were inoculated with mycelia plugs (7 mm in diameter), separated from the margin of 5-days old culture. Five Petri dishes (diameter 9 cm) of each isolate were incubated in conditions described above. The diameter of colonies was measured 5 days after inoculation and used to calculate the isolate growth rate (mm/day). The isolates were divided into two groups according to growth rate. Group A involved isolates with lower and average growth rate (average growth rate for *F. avenaceum*: 15°C – 2.3 mm/day, 25°C –3.8 mm/day; *F. culmorum*: 15°C – 5.6 mm/day, 25°C – 12.8 mm/day; *F. graminearum*: 15°C – 4.2 mm/day, 25°C – 10.2 mm/day; *M. nivale*: 15°C – 9.4 mm/day, 25°C – 5.1 mm/day; *F. poae*: 15°C – 7.2 mm/day, 25°C – 10.2 mm/day), Group B involved isolates with growth rate higher than the mean calculated from growth rates of all tested isolates (Brennan *et al.* 2003).

Pathogenicity test. The test was carried out on wheat kernels (fraction above 2.5 mm) of cultivar Brea (germinative capacity 99.50%). The kernels were surface sterilised by shaking in 1% NaOCl solution for 2 minutes. Next the seeds were rinsed in redistilled water and air dried. The same amount of PDA (30 ml) was dosed in each "testing" Petri dish (TPD). For the inoculation of TPD (diameter 9 cm) 5-days-old cultures of each isolate were used (20 isolates of each species and location in 5 replications). The mycelium-agar plugs (7 mm diameter) separated from the margin of the inoculation subcultures were used for inoculation. TPD were incubated under the conditions described above. Isolates were ready for the pathogenicity test when their mycelium overgrown

Figure 1. Sampled locations of Slovakia

Table 1. Characterisation of sampled locations

Locality	Altitude (m)	Average year rainfall rate (mm)	Average year temperature (°C)
Sládkovičovo*	122	497.2	10.46
Želiezovce*	137	588	9.4
Bučany*	165	570	9.4
Nitra*	180	539	10.2
Malý Šariš**	295	599.3	7.86
Vígľaš-Pstruša**	375	640	7.9

*plain regions; **mountains regions

the whole surface of the agar plate. Consequently 10 surface-sterilised wheat kernels were placed on the mycelium surface in TPD. The kernels were lightly pressed to contact with agar (moisture for germination) by pressing down the aerial mycelium. The TPD with kernels were incubated 10 days at 15°C and 25°C. Control variants were prepared in the same way, but without mycelium inoculation. The average number of infected roots was counted and presented as percentage of infected roots. The number of germinated and ungerminated kernels was expressed as germination retardation (%). The overgrown biomass was measured on the tenth day and expressed as average weight of separated kernels, roots and sprouts and calculated as biomass growth retardation (Brennan et al. 2003). The obtained results were tested by analysis of variance, Tukey's test, $P = 0.05$.

RESULTS

Influence of temperature and origin on growth rate

Irrespective of the origin, the growth of *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, and *F. poae* was the fastest at 25°C, of *M. nivale* at 15°C (Table 2). *F. graminearum* and *F. poae* showed very similar growth rates at 25°C, but at 15°C *F. poae* grew faster than F. *graminearum*. Of all the tested species, *F. avenaceum* was the slowest-growing species at both temperatures.

In the *F. avenaceum* and *M. nivale* isolates, there were not any significant differences between the growth rates of isolates of different origin. The *F. culmorum*, *F. graminearum*, and *F. poae* isolates originating from mountain regions (MR) grew significantly faster at 15°C than those from flatland regions (FR). At the temperature of 25°C, the FR isolates grew significantly faster than those from MR.

Pathogenicity of isolates to wheat seedlings

The *in vitro* test was used to examine the effect of *Fusarium* and *M. nivale* isolates on the germination, root development and coleoptile and biomass growth of wheat seedlings (Figure 2).

F. culmorum and *F. graminearum* were the most pathogenic to the root development at both cultivation temperatures, while *M. nivale*, *F. poae* and *F. avenaceum* were less pathogenic. The pathogenicity to germination retardation was assessed in descending order: *F. culmorum*, *F. graminearum*, *M. nivale*, *F. avenaceum*, *F. poae*. There is a great variability in biomass growth retardation at both cultivation temperatures. The species pathogenicity at 15°C was assessed in descending order: *F. culmorum*, *F. graminearum*, *M. nivale*, *F. avenaceum*, *F. poae*; at 25°C as follows: *F. graminearum*, *F. culmorum*, *F. avenaceum*, *M. nivale*, and *F poae*.

The effect of *Fusarium* spp. and *M. nivale* inoculation on evaluated parameters (percentage of infected roots, germination retardation, and biomass growth retardation) was analysed with respect to the origin of the isolates. There were no significant differences in the pathogenicity of *F. avenaceum* and *F. graminearum* isolates from different localities. Higher pathogenicity at 25°C than at 15°C was determined.

The same pathogenicity to roots was assessed at both temperatures in *F. culmorum* isolates. Higher pathogenicity to germination retardation and biomass growth retardation was found out at 15°C in all isolates of *F. culmorum*.

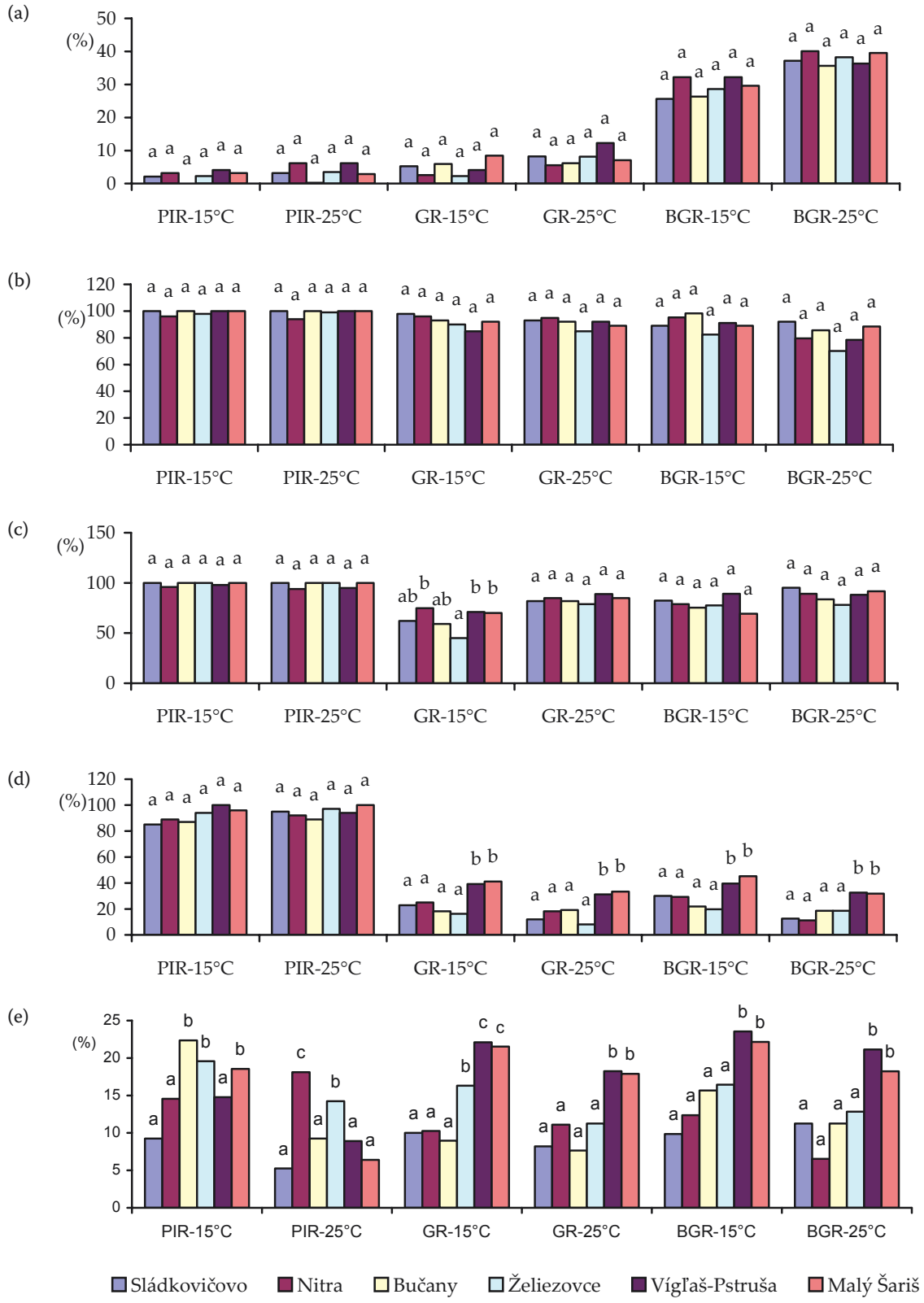
Table 2. *In vitro* growth rate of *Fusarium* spp. and *Microdochium nivale* isolates

Species	Locality origin of isolates	Number of isolates per GRG at			
		15°C		25°C	
		A	B	A	B
F. avenaceum *15°C – 2.3 mm/day *25°C –3.8 mm/day	Sládkovičovo	11[a***]	9[a]	13[a]	7[a]
	Bučany	10[a]	10[a]	8[a]	12[a]
	Malý Šariš	9[a]	11[a]	10[a]	10[a]
	Vígľaš-Pstruša	8[a]	12[a]	11[a]	9[a]
	Nitra	7[a]	13[a]	9[a]	11[a]
	Želiezovce	9[a]	11[a]	10[a]	10[a]
F. culmorum *15°C – 5.6 mm/day *25°C – 12.8 mm/day	Sládkovičovo	15[b]	5[a]	7[a]	13[b]
	Bučany	13[b]	7[a]	8[a]	12[b]
	Malý Šariš	4[a]	16[b]	15[b]	5[a]
	Vígľaš-Pstruša	6[a]	14[b]	16[b]	4[a]
	Nitra	12[b]	8[a]	7[a]	13[b]
	Želiezovce	13[b]	7[a]	6[a]	14[b]
F. graminearum *15°C – 4.2 mm/day *25°C – 10.2 mm/day	Sládkovičovo	14[b]	6[a]	7[a]	13[b]
	Bučany	12[b]	8[a]	8[a]	12[b]
	Malý Šariš	11[b]	9[a]	14[b]	6[a]
	Vígľaš-Pstruša	7[a]	13[b]	9[a]	11[b]
	Nitra	13[b]	7[a]	8[a]	12[b]
	Želiezovce	14[b]	6[a]	7[a]	13[b]
M. nivale *15°C – mm/day *25°C – mm/day	Sládkovičovo	8[a]	12[a]	11[a]	9[a]
	Bučany	9[a]	11[a]	8[a]	12[a]
	Malý Šariš	10[a]	10[a]	11[a]	9[a]
	Vígľaš-Pstruša	9[a]	11[a]	8[a]	12[a]
	Nitra	7[a]	13[a]	9[a]	11[a]
	Želiezovce	6[a]	14[a]	11[a]	9[a]
F. poae *15°C – mm/day *25°C – 10.2 mm/day	Sládkovičovo	16[b]	4[a]	7[a]	13[b]
	Bučany	13[b]	7[a]	6[a]	14[b]
	Malý Šariš	5[a]	15[b]	13[b]	7[a]
	Vígľaš-Pstruša	6[a]	14[b]	18[b]	2[a]
	Nitra	13[b]	7[a]	6[a]	14[b]
	Želiezovce	14[b]	6[a]	7[a]	13[b]

GRG – Growth Rate Group; A – isolates with lower and average growth rate; B – isolates with higher growth rate than average, *average growth rate of species isolates at the described temperature; differences between values (within species-localities groups) designated by the same letter are not significant (analysis of variance, Tukey's test, $P = 0.05$)

The MR isolates of *M. nivale* and *F. poae* were significantly more pathogenic than FR isolates. The pathogenicity of all isolates was higher at 15°C than at 25°C, except the roots which were more damaged at 25°C. All *M. nivale* isolates were more pathogenic than those of *F. poae*.

The results suggest that the species and origin of isolates were the most significant factors affecting pathogenicity and that they had significant effects at both temperatures. There could be many relationships which might exist between the *in vitro* growth rate and pathogenicity of the species isolates.



PIR – percentage of infected roots; GR – germination retardation; BGR – biomass growth retardation differences between values designated by the same letter are not significant (analysis of variance, Tukey's test, $P = 0.05$)

Figure 2. Effect of temperature on the pathogenicity of *F. avenaceum* (a), *F. culmorum (b), F. graminearum* (c), *M. nivale* (d), and *F. poae* (e) isolates

DISCUSSION

This experiment was conducted to determine the influence of temperature on the pathogenicity of *Fusarium* and *Microdochium* isolate collection under *in vitro* conditions. A similar method for the *in vitro* growth and pathogenicity measurement was used previously (Mesterházy 1984; Clear & Patrick 2000; Brennan *et al.* 2003). The test of *Fusarium graminearum* pathogenicity to wheat seedlings can be used for the prediction of head blight development via seedling test, for other *Fusarium* species the relationship between the *in vitro* test and FHB has not been known yet (Mesterházy 1984).

The results of the growth rates of tested *Fusarium* and *Microdochium* species are in agreement with results obtained by other authors (Gerlach & Nirenberg 1982; Brennan *et al.* 2003). The growth rates of *F. culmorum*, *F. graminearum*, *F. poae*, and *F. avenaceum* are higher at 25°C than at 15°C. Similar results were reported by some authors (Pettit *et al.* 1996; Campbell & Lips 1998; Brennan *et al.* 2003) who found out that the optimal temperature for *F. culmorum*, *F. graminearum* and *F. avenaceum* was between 20°C and 25°C, while *M. nivale* grew better at temperatures lower than 20°C. In general, *M. nivale* and *F. culmorum* are known as species that able to grow at low temperatures (Pitt & Hocking 1997; Doohan *et al.* 2003).

The pathogenicity test highlighted the variation among species and isolates in germination, root development and biomass growth retardation at different temperatures. The highest pathogenicity of tested species to seedling roots was recorded in *F. culmorum*, *F. graminearum* and *M. nivale* at both temperature and the lowest in *F. avenaceum* and *F. poae*. These results are in contrast with the results of Ponchet (Ponchet 1966), who reported that *M. nivale* in a glasshouse study was non-pathogenic to seedlings above 16°C. Our results correspond with Brennan *et al.* (2003), who reported the highest pathogenicity of *M. nivale* to wheat seedlings at lower temperatures. The analysis of presented results shows that the species and origin factors were the most important contribution to the variation observed in both *in vitro* growth rates and seedling pathogenicity of *M. nivale* and *F. poae*. This conclusion is in agreement with previously reported results showing that different *Fusarium* species varied in their growth and pathogenicity (Brennan *et al.* 2003). The test also showed the variation of *F. culmorum* and *F. poae* isolates in the growth rate due to their origin. The MR isolates grew significantly faster at lower temperatures and were significantly more pathogenic in germination and biomass growth retardation than the FR isolates. The differences in growth rate and pathogenicity are probably influenced by specific weather conditions in the study sites and consecutive adaptation of isolates to different conditions (Bottalico & Perrone 2002; Doohan *et al.* 2003). According to results it seems that the isolates probably formed ecotypes (Pettit *et al.* 1996). Similar results were obtained by Brennan *et al.* (2003), who reported growth rate variability in the European *Fusarium* species collection. He recognized that the isolates originating from some regions were not able to cause the same infection levels in different agroecological conditions, except possible adaptation and regaining the former pathogenicity. Otherwise, the variability of species and isolates indicates that the pathogen-host interaction is highly complex (Doohan *et al.* 2003) due to the existence of indirect (Walker *et al.* 2001) and direct relationship between *in vitro* growth and *in vivo* pathogenicity (Bai & Shaner 1996). There is a consensus that the true host specificity does not exist in *Fusarium* species, but variation in aggressiveness among their isolates has been documented (Miedaner & Schilling 1996; Miedaner *et al.* 2004). It follows from this analysis that the species and origin of isolates were the most significant factors affecting the pathogenicity of *Fusarium* and *M. nivale* isolates and had significant effects at both temperatures. There could be many relationships which might exist between the *in vitro* growth rate and pathogenicity of the species isolates.

The present study highlighted the importance of isolate origin and incubation temperature as important factors for the growth and seedling pathogenicity of selected species. The results suggest that not only within Europe (Brennan *et al.* 2003) but also within the countries the temperature ecotypes of *Fusarium* spp. *Microdochium nivale* may exist. This knowledge could be interesting for specialised breeding programmes in different agroecological conditions in the future.

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