

Correlation between Black Point Symptoms and Fungal Infestation and Seedling Viability of Wheat Kernels

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Abstract

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The level of occurrence of black point, the spectrum of fungal species and damage to wheat seedling vigour associated with it were assessed during 2003 and 2004 in the Slovak Republic. The incidence of black point kernels ranged between 0.2–2.4% in 2003 and 24.2–34.3% in 2004. The kernels' fungal contamination varied from 60% to 100%. *Alternaria* spp., *F. poae* and *F. culmorum* were isolated from all localities and all subsamples. *Stemphylium vesicarium*, *Fusarium culmorum*, *F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, *Microdochium nivale*, *Epicoccum nigrum*, *Penicillium* spp., *Aspergillus niger*, *Rhizopus nigricans* and *Cochliobolus sativus* were isolated less frequently. Fungi of the genus *Alternaria* were the most dominant, followed by *Fusarium* and *Microdochium* among which *F. poae* was dominant. Irrespective of incubation temperature, the germinative capacity and coleoptile growth rate of discolored kernels were affected more in the wet and cold year 2004. The inhibition of germination and seedling viability was more pronounced at the incubation temperature 22°C than at 15°C. Inhibition of coleoptile growth rate was 0.12–3.12% in black point kernels collected in 2003, and 0.24–9.28% in those collected in 2004.

Keywords: wheat; kernels; black point; *Fusarium*, *Alternaria*; germination

An important factor responsible for low productivity of wheat is poor seed germination and early seedling mortality due to seed-borne fungi. The host and climatic factors influence the growth, survival, dissemination and hence the incidence of seed borne fungi and the disease severity. The influence of locality, host cultivars, pathogenicity and toxicity of these fungi were extensively studied (MIEDANER *et al.* 2001; MAGG *et al.* 2002). The influence of climatic factors on the occurrence of seed borne fungi is complicated by the fact that the fungi can infect or cause disease individually or as a complex (DOOHAN *et al.* 1998). There are numerous reports on how the fungal species

respond differently to environmental variations, especially temperature and humidity. However, host susceptibility to fungal disease is directly influenced by temperature, humidity and osmotic stress (CONRATH *et al.* 2002). Consequently, *Fusarium* spp. and *Alternaria* spp. inflict yield losses in the field and cause significant decrease in wheat quality that can go as far as to render the harvested grain useless for processing (SARIC *et al.* 1997).

Black point symptoms on wheat kernels appear in some years that are characterised by frequent rainfall at time of seed formation, maturation and premature seed senescence. Diseased kernels are discolored, withered, black-pointed or

smudged, with darkened pericarp and shrivelled embryo. The germination of black point kernels is decreased and these discolored grains are undesirable for the market and food industry (WIESE 1987). From a certain incidence of kernel discoloration on, the harvested grain is usually downgraded (WANG *et al.* 2003). In case of black point, the location–cultivar–year interaction is a significant source of variation and a crossover cultivar–environment interaction was significant, suggesting that rank order of cultivars differed with the environment (FERNANDEZ *et al.* 2000). Several seed-borne fungi, including species of the genera *Alternaria*, *Fusarium*, *Aspergillus* and *Penicillium* have been considered as important pathogens of cereal grains (HASSAN 1999; DOOHAN *et al.* 2003). Species of *Alternaria*, *Fusarium*, *Cochliobolus*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Rhizopus* and *Stemphylium* are associated with black point symptoms (WIESE 1987). *Alternaria* species are parasitic on plants and other organic materials. *Alternaria alternata* is a frequently occurring species; it produces a number of mycotoxins, including alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxins I, II, and III (ATX-I, -II, and -III) and L-tenuazonic acid (TeA) (SCOTT 2001; LI *et al.* 2001).

The objectives of the present work were to evaluate the influence of the growing season on the level of occurrence of black point kernels, to identify the associated fungal species, and to determine the correlation between the frequency of black point symptoms and the level of damage to the viability of wheat seeds in the Slovak Republic.

MATERIALS AND METHODS

Sample collecting

Samples of winter wheat seed (1 kg per sample) were collected at four localities (Nitra, Sládkovičovo, Želiezovce and Víglaš-Pstruša) after standard har-

vest in the years 2003 and 2004. Subsamples divided to kernels with black point symptoms (BPK) and asymptomatic ones (AK) (300 seeds per subsample) were used for the experiments. Table 1 lists the locations where samples were taken; they were four state experimental stations, representing different climatic conditions of the Slovak Republic.

Isolation and identification of the fungi

Seeds from the symptomatic and asymptomatic subsamples were surface-sterilised by shaking for 2 min in 1% NaOCl solution, and rinsed with redistilled water. The seeds were placed in Petri dishes containing potato-dextrose agar (PDA) and these were incubated at 22°C under 12/12 photoperiod (4000 lux, no UV light). Subcultures were isolated from mature fungal colonies, further purified and identified by visual and microscopic observation of single spore cultures.

Synthetic nutrient agar (SNA), PDA, UV-light (365 nm) and incubation at 22°C under 12/12 photoperiod (4000 lux, no UV light) were used for culture and identification of *Fusarium* subcultures. Species were identified by referring to manuals and a monograph (NIRENBERG 1981; NELSON *et al.* 1983; SAMSON *et al.* 2002).

Potato carrot agar (PCA) was used to grow and identify by sporulation pattern and conidia morphology the subcultures of the genus *Alternaria* (SIMMONS & ROBERTS 1993; ANDERSEN *et al.* 2001) and of other genera and species (SAMSON *et al.* 2002).

Determination of germinative capacity

Hundred kernels per subsample (in five repetitions) were placed on moistened filter paper in a “germinative” Petri dish (50 seeds per dish, $d = 20$ mm), incubated at 22°C and 12/12 photoperiod, and after 10 days the percentage of germinated and ungerminated kernels was calculated.

Table 1. Climatic conditions at sampled localities

Locality	Altitude (m)	Annual mean rainfall (mm)	Annual mean temperature (°C)
Nitra	180	539	10.2
Sládkovičovo	122	497.2	10.46
Želiezovce	137	588	9.4
Víglaš-Pstruša	375	640	7.9

Determination of retardation of coleoptile growth rate

The test was done on wheat kernels with black point symptoms according to a modified method of BRENNAN *et al.* (2003). The kernels were surface-sterilised by shaking for 2 min in 1% NaOCl solution, rinsed in redistilled water and air dried. Ten wheat kernels were lightly pressed into contact with the surface of PDA (30 ml) in each “testing” Petri dish (TPD); the dishes were incubated at 15°C and 22°C (20 plate/location/subsample). The length of the coleoptile measured after 10 days was used to determine the growth rate (mm/day); the results were expressed as percentage of coleoptile growth rate retardation relative to a control test with asymptomatic kernels.

The results were tested by Analysis of variance, Tukey test, $P = 0.05$.

RESULTS

Spectrum of fungal species of black point kernels

The numbers of isolated species and total fungal contamination of both subsamples (asymptomatic kernels – AK, and kernels with black point symptoms – BPK) are shown in Figures 6–8. All samples showed a high level of fungal contamination, varying from 60% to 100% (Figure 1). There was a significantly higher occurrence of BPK and a higher total infestation in the BPK and AK subsamples collected in 2004 (especially at the localities Nitra, Sládkovičovo and Želiezovce). The significant in-

fluence of the year on BPK occurrence and total kernel infestation was confirmed by the results.

Table 2 shows the level of contamination by the different fungi in the subsamples BPK and AK. Only *Alternaria* species, *F. poae* and *F. culmorum* were isolated from all localities and subsamples. *Alternaria* species were the most dominant (infestation ranged from 46.2% to 90.2 %), followed by *Fusarium* spp. The highest incidence of *Alternaria* spp. was recorded in BPK subsamples in the year 2004 and at all localities, except Víglaš-Pstruša. *Fusarium poae* was the most frequent *Fusarium* species, it ranged from 3.5% to 15.7%. The total kernel infestation by fungi other than the fungal genera *Alternaria* and *Fusarium* ranged from 0.06% to 12.7%. Among them were *Stemphylium vesicarium*, *Fusarium culmorum*, *F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, *Microdochium nivale*, *Epicoccum nigrum*, *Penicillium expansum*, *Aspergillus niger*, *Rhizopus nigricans* and *Cochliobolus sativus*; their individual incidence did not exceed 7.1%. Some species were isolated sporadically only from one subsample or only from some of the sampled localities.

Occurrence of black point symptoms on seeds and its association with seedling viability

In the tests, the deleterious effect of black pointed kernels on germination and development of the seedling, expressed as coleoptile growth rate retardation (CGR), were determined. The CGR was analysed with respect to the kernels origin and year of collecting (Figures 2 and 3). Large differences in BPK occurrence in different years were

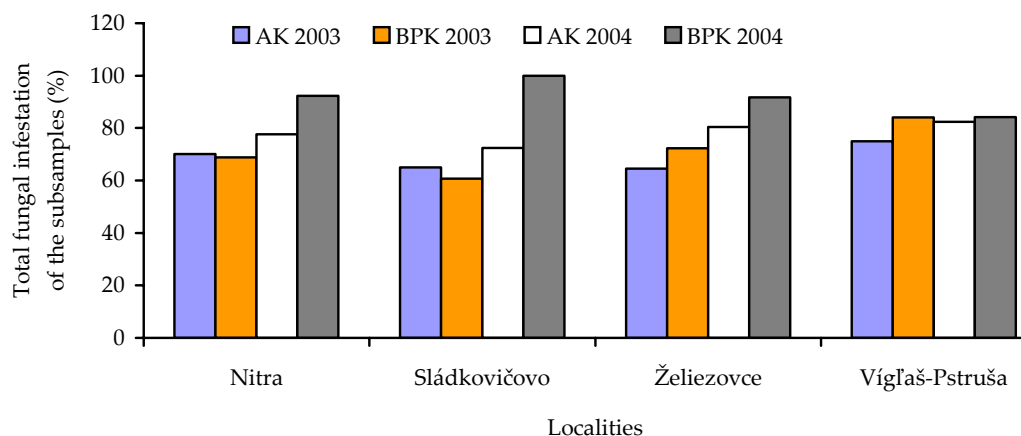


Figure 1. Total fungal colonisation of the subsamples (AK – asymptomatic kernels, BPK – kernels with black point symptoms)

Table 2. The occurrence of fungal species in the BPK and AK subsamples (%)

Locality	Year	Kernel type and percent in sample	ASP	FP	FC	FG	FA	FS	MN	SV	EN	PE	AN	RN	CS
Nitra	2003	BPK (0.2 ^a)	60.64	3.21	0.1	1.2	1.23	0.01	0.0	0.0	2.1	0.02	0.32	0.0	0.0
		AK (99.8 ^d)	61.5	5.2	0.5	0.2	0.4	1.2	0.0	0.0	0.0	0.0	1.0	0.0	0.0
	2004	BPK (29 ^b)	74.3	8.6	1.3	1.1	2.3	0.0	0.0	2.2	1.4	1.1	0.0	0.0	0.0
		AK (71 ^c)	51.4	11.6	5.7	2.9	1.4	1.6	0.0	0.0	0.1	2.9	0.0	0.0	0.0
Sládkovičovo	2003	BPK (1.23 ^a)	59.0	1.0	0.2	0.0	0.1	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
		AK (98.77 ^d)	61.0	3.5	0.4	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0
	2004	BPK (24.2 ^b)	90.2	8.0	0.5	0.0	0.0	0.0	0.0	1.0	2.0	0.0	0.0	0.0	0.0
		AK (75.8 ^c)	46.2	7.7	4.6	1.5	0.2	0.0	4.6	1.5	0.0	1.2	1.3	0.4	3.2
Želiezovce	2003	BPK (1.01 ^a)	63.8	4.2	2.1	0.4	1.4	0.2	0.1	0.0	0.0	0.0	0.1	0.0	0.0
		AK (98.99 ^d)	55.2	5.2	0.2	0.0	2.3	0.0	0.0	0.0	0.5	0.0	0.0	0.0	1.0
	2004	BPK (29.3 ^b)	82.2	6.0	0.3	0.3	0.6	1.2	0.0	0.0	1.2	0.0	0.0	0.0	0.0
		AK (70.7 ^c)	64.3	7.1	0.2	1.1	0.3	0.0	0.0	2.8	0.1	0.0	0.1	0.0	4.3
Víglaš-Pstruša	2003	BPK (2.35 ^a)	69.8	8.2	3.1	2.0	0.2	0.2	0.2	0.2	0.0	0.4	0.0	0.0	0.0
		AK (97.65 ^d)	58.5	9.4	1.1	0.0	1.7	2.3	1.6	0.1	0.0	0.0	0.0	0.0	0.3
	2004	BPK (34.26 ^b)	61.7	5.1	0.2	1.3	2.1	1.1	0.0	0.2	3.2	2.1	7.1	0.0	0.0
		AK (65.74 ^c)	52.3	15.7	2.4	2.2	3.6	1.0	0.0	1.5	2.0	0.0	0.2	1.5	0.0

BPK – kernels with black point symptoms, AK – asymptomatic kernels, ASP – *Alternaria* spp., SV – *Stemphylium vesicarium*, FP – *Fusarium poae*, FC – *F. culmorum*, FG – *F. graminearum*, FA – *F. avenaceum*, MN – *Microdochium nivale*, EN – *Epicoccum nigrum*, PE – *Penicillium expansum*, FS – *F. sporotrichioides*, AN – *Aspergillus niger*, RN – *Rhizopus nigricans*, CS – *Cochliobolus sativus*

Percentage of BPK or AK in the samples, differences between values assigned the same letter are not significant (Analysis of variance, Tukey test, $P = 0.05$)

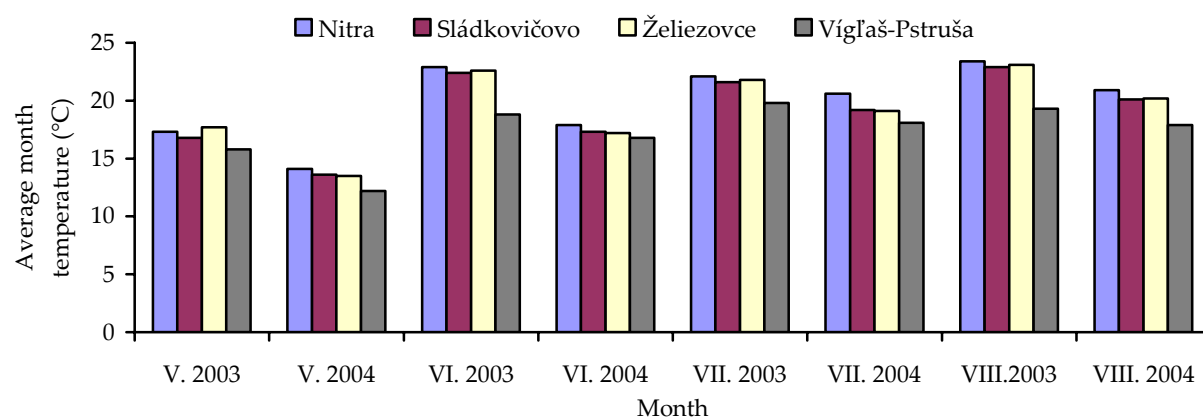


Figure 2. Monthly mean temperature at sampled localities in the years 2003 and 2004

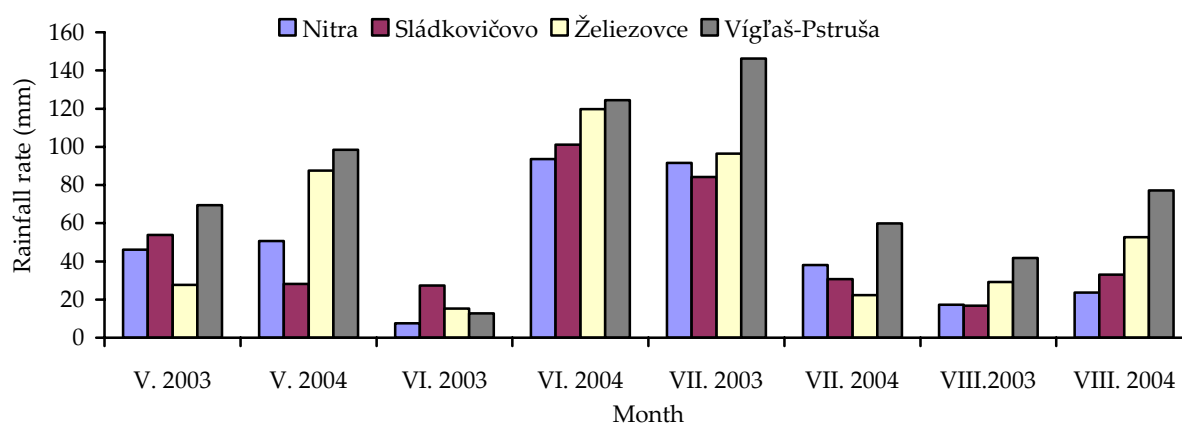


Figure 3. Monthly rainfall rate at sampled localities in the years 2003 and 2004

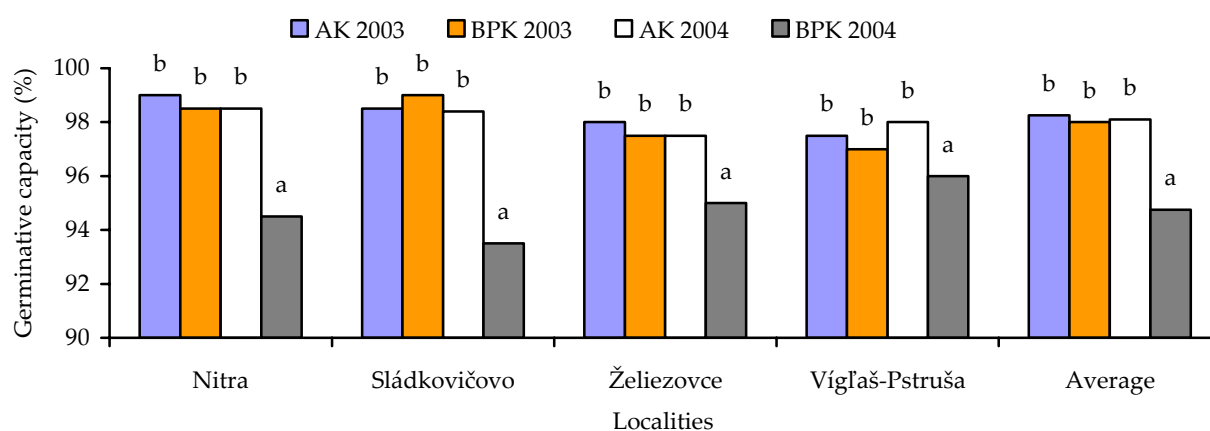


Figure 4. Germinative capacity in selected subsamples

AK – asymptomatic kernels, BPK – kernels with black point symptoms; differences between values signed by the same letter are not significant (Analysis of variance, Tukey test, $P = 0.05$)

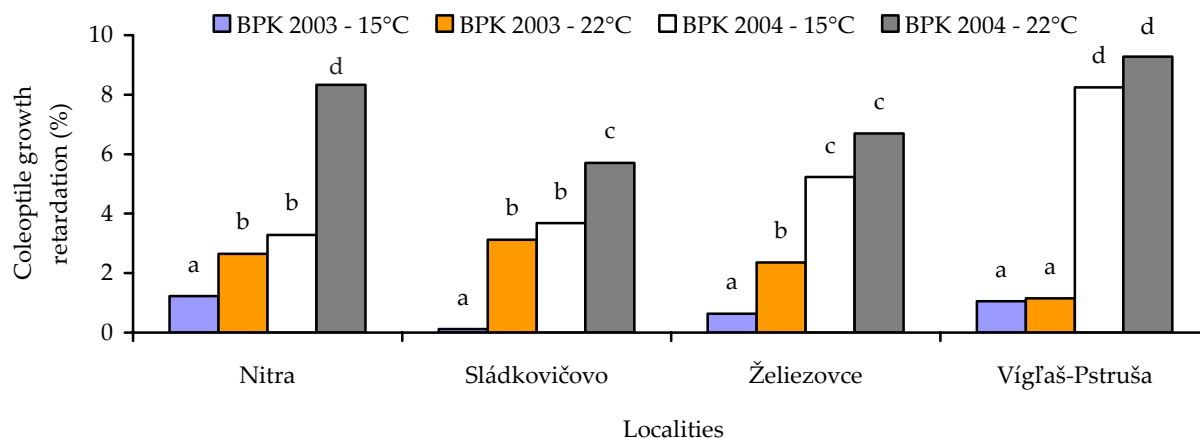


Figure 5. Coleoptile growth retardation of “black point kernels” subsamples

BPK 2003 – 15°C – kernels with black point symptoms, year 2003, cultured at 15°C; differences between values signed by the same letter are not significant (Analysis of variance, Tukey test, $P = 0.05$)

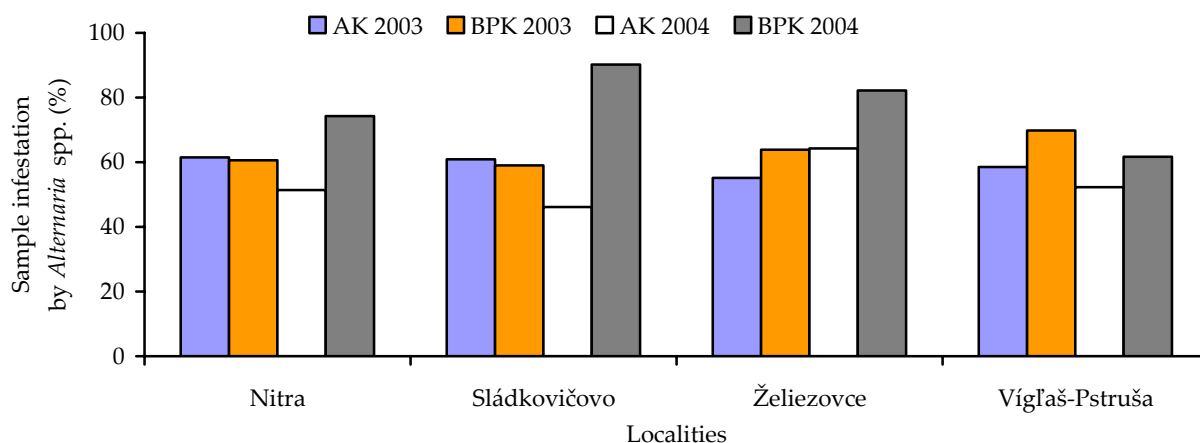


Figure 6. Sample of infestation by *Alternaria* spp. (AK – asymptomatic kernels, BPK – kernels with black point symptoms)

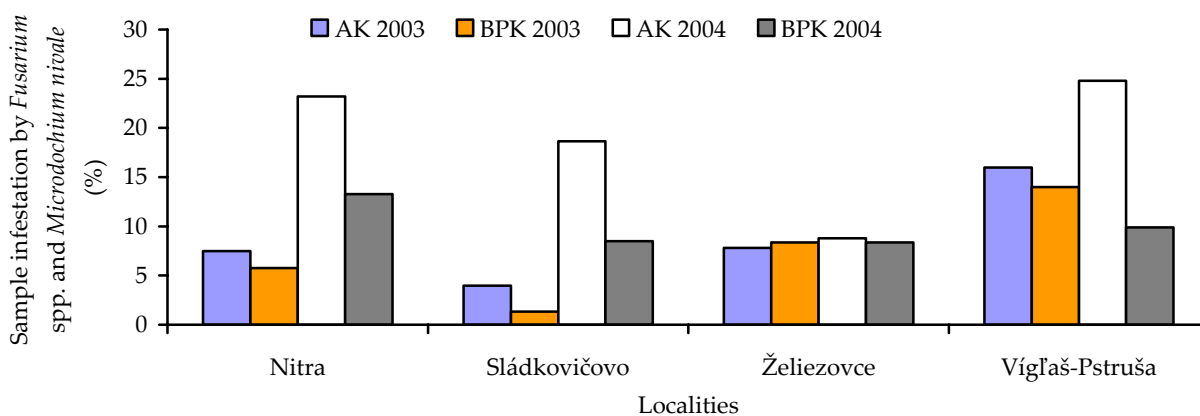


Figure 7. Sample of infestation by *Fusarium* spp. and *Microdochium nivale* (AK – asymptomatic kernels, BPK – kernels with black point symptoms)

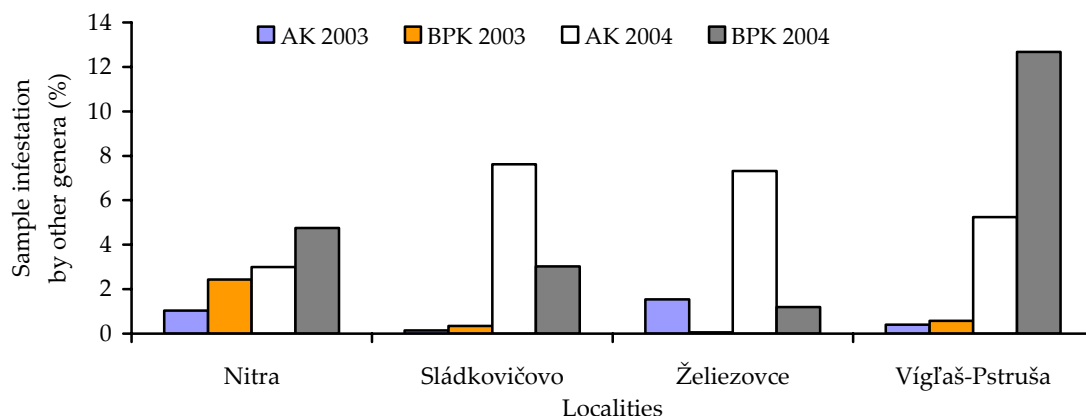


Figure 8. Sample of infestation by other fungal genera (AK – asymptomatic kernels, BPK – kernels with black point symptoms)

recorded. The occurrence of BPK in samples from the four locations ranged from 0.2% to 2.35% in the year 2003, and from 24.2% to 34.26% in 2004 (Table 2).

Figures 4 and 5 depict the correlation of black point symptoms with seedling viability. Irrespective of incubation temperature, the germinative capacity and coleoptile growth rate of BPK were affected more in seeds harvested in 2004. Analysis of CGR at different incubation temperatures showed significant differences between 15°C and 22°C. In 2004, the inhibition of grain emergence and seedling viability was more pronounced at the incubation temperature of 22°C than at 15°C. The CGR in seedlings of BPK subsamples ranged from 0.12% to 3.12% in the year 2003, and from 0.24% to 9.28% in 2004, relative to AK control seedlings.

DISCUSSION

Locality and time of sampling influenced the occurrence of BP symptoms and fungal infestation of the kernels. They are important indicators for general recommendations for agricultural practice. The significantly higher black point occurrence and kernel infestation in the colder and moister year 2004 are useful data for practical plant protection measures. The results are in agreement with those obtained by WIESE (1987) who mentioned high black point occurrence in humid environment during rainfall at the time of seed formation, maturation and premature seed senescence. The high black point severity on the wheat kernels might have been due to cool, wet weather conditions

and frost during seed development that delayed ripening (FERNANDEZ *et al.* 2000). In the USA, a level of 2–4% BPK is permitted in wheat samples for the food industry (WIESE 1987). According to our results, some of the wheat samples collected in the year 2003 were just below this limit, while the samples from 2004 were over the limit. The results confirmed that the microclimate of a locality is more important for BPK occurrence than the general agroclimatic conditions of an evaluated year.

The observed levels of kernel infestation by *Fusarium* spp. correspond with the results of YLIMATTILA *et al.* (2002), who found an infestation in wheat grain of 4–20% by this genus. The dominance of *F. poae* among *Fusarium* species is not in agreement with results of PROKINOVÁ (1999) who considered *F. poae* as a less frequent species in black point kernels of barley. On the other hand, *F. poae* is the most frequent species in asymptomatic wheat kernels in the Slovak Republic (HUDEC & ROHÁČIK 2003). The presumption of increasing incidence of fungal pathogens (including *Fusarium* species in grains above 2.5 mm) in moister locations (ELEN *et al.* 2000) is confirmed by the present results. This kernel fraction is used as seed for sowing in agricultural practice. The infestation of these asymptomatic kernels occurs during their formation. Since in our tests the saprophytic mycoflora on the surface of the kernels was eliminated by treatment with NaOCl, the species were harboured underneath the surface tissues of evaluated grains. These sources of inoculum are important for further infections and epidemiology in the field (WIESE 1987). The results suggest that wet years (e.g.

2004) are favourable for black point occurrence and fungal infestation, especially high rainfall rate and cool temperature throughout May and June, which agrees with results of PROKINOVÁ (1999).

The spectrum of isolated fungal species is in agreement with WIESE (1987), who mentioned many fungal species associated with BP symptoms, including species isolated in this study. In the year 2004, a high incidence of *Fusarium* head blight (FHB) was observed in the Slovak Republic at all investigated localities (unpublished information). It may suggest that in a year of a FHB epidemic there may also be a high incidence of BPK. Despite these reports, our results showed *Alternaria* spp. as the predominant species in the BPK subsamples, not *Fusarium* species. It suggests that there is no correlation between a FHB epidemic, kernel infestation by *Fusarium* fungi and BPK occurrence. The fungi isolated from BPK can co-parasitise seed but differ widely in aggressiveness. Without competition from other micro-organisms, each tends to dominate the substrate (WIESE 1987). The highest frequency of *Alternaria* spp. in BPK suggests these species to be the most important contributors of BP symptoms formation. In both oat and wheat seeds, *Alternaria* spp. are the most frequent species (CLEAR *et al.* 2001; HUDEC & ROHÁČIK 2003). This also agrees with the results of PROKINOVÁ (1999) that showed a major role by *Alternaria* spp. and *Bipolaris sorokiniana* in the occurrence of black point symptoms in barley kernels.

The common fungal species frequently associated with wheat kernels were screened to assess their ability to induce pre- and post-harvest mortality using a kernel infestation method. The pathogenic fungi, associated with black point symptoms of wheat kernels induced variable reduction of seedling viability. Although seed damage from black point begins before harvest, it increases if grains are stored under moist or wet conditions (WIESE 1987). According to our results, the seedling vigour was most affected in kernels harvested in the year 2004, which was characterised by lower temperatures and higher rainfall rate than in the previous year (mainly during May and June). In 2004, a higher infestation of kernels by saprophytic and phytopathogenic fungi was recorded. Higher fungal infestation probably co-influenced the seedling vigour together with another agro-environmental factors. In general, species from the *Alternaria*, *Penicillium*, *Aspergillus* and *Fusarium* genera and *Cochliobolus sativus* isolated from BPK

are capable of killing or reducing the growth of cereal seedlings, and have the ability to produce their respective toxins (HASSAN 1999). Seed-borne fungi may injure the embryo by producing some toxins which result in germination failure or may reduce seedling vigour in case of germination (HASSAN 1999). *Alternaria alternata* is a commonly occurring species of particular interest because it produces a number of mycotoxins, including alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxins I, II, and III (ATX-I, -II, and -III) and L-tenuazonic acid (TeA) (LI *et al.* 2001; SCOTT 2001).

The results indicated *F. poae* to be the most frequent *Fusarium* species. The species is not a dangerous pathogen for germinating wheat kernels (HUDEC & BOKOR 2003), but under optimal conditions is able to decrease kernel germination and coleoptile formation (BRENNAN *et al.* 2003). On the other hand, *F. poae* is a dangerous producer of mycotoxins (NIV, ZEA, DAS, FUS, ENS, HT-2 tox. and T-2 tox.) (NELSON *et al.* 1983) and occurs frequently in Northern and Central Europe (BOTTALICO & PERRONE 2002). Some authors reported a direct correlation between the occurrence of toxigenic *Fusarium* species and mycotoxins production (SNIJDERS & PERKOWSKI 1990), but usually no clear correlation was found between species composition and mycotoxins in grains (YLI-MATTILA *et al.* 2002).

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