Crown Rust Pathotypes Determined on Oats in the Czech Republic from 2004 to 2006 and Reaction to Oat Cultivars

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Abstract: The incidence of oat crown rust (*Puccinia coronata var. avenae* Fraser *et* Ledingham) pathotypes was recorded in the Czech Republic in 2004–2006. The virulence of collected 79 monopustule oat crown rust isolates was assessed on 23 differentials. High diversity was observed; the isolates possessed from 0 to 8 virulence genes. More than 80% of pathotypes were recorded only once. Pathotypes BLBG and BLBC were the most frequent. None of the tested isolates was virulent to resistance genes *Pc39, Pc50, Pc52, Pc59, Pc62* and *Pc68*. Registered cultivars Avenuda, Dalimil, Auron, Isak were susceptible to the majority of pathotypes, cvs. Abel, Ardo, Atego, Azur, Neklan, Radius and Saul to all used pathotypes. Only the Czech cultivar Vok was resistant to all but four used pathotypes. Acquired data are useful for resistance breeding.

Keywords: oat; crown rust; cultivar resistance; *Pc* genes; *Puccinia coronata* var. *avenae*; pathotypes; Czech Republic

In recent years oat grains have become a more significant component in the human diet, particularly due to the favourable content of amino acids, fatty acids and β-glucans contained in the fibre which has been shown to lower risks of civilization diseases (Truswell 2002). In 2002–2007 oat hectarage varied between 51 567 and 77 371 in the Czech Republic.

Oat crown rust, incited by *Puccinia coronata* var. *avenae*, has been considered the most important fungal disease of cultivated oat (*Avena sativa* L.) in many regions of the world (Simons 1985; Harder & Haber 1992) including the Czech Republic. Regular occurrences of the oat crown rust epidemics have been recorded since the beginning of the disease surveys not only in Europe (Šebesta *et al*. 2003) but also in Africa (Niekerk *et al*. 2001), North and South America (Kolmer & Chong 1993; Chong & Zegeye 2004; Leonard & Martinelli 2005).

A composite life cycle of *Puccinia coronata* var. *avenae*, involving both sexual and asexual propagation associated with host alternations, has already been well described as well as the ability of rust spores to migrate for very long distances by wind (Hovmøller *et al*. 2002). According to Urban (1969) the intermediate host *Rhamnus cathartica* plays a less important role in Europe than in North America; close vicinity with oat fields seems to be important for infection with crown rust.

It is widely accepted that the breeding for crown rust resistance is the most economic and ecologic way to control the disease (Chong & Brown 1996). Such breeding programmes are the most effective when associated with monitoring of virulence of the pathogen populations. The main purpose of such surveys is to estimate the relative prevalence and distribution of virulence phenotypes and to detect shifts toward virulence to resistance genes being used (Chong & Zegeye 2004).

Although techniques of molecular biology have been used for the assessment of genetic variability of oat crown rust populations, most information on the pathogen physiologic specialisations and identification of pathotypes in relation to resistance breeding has been acquired by the analyses

of virulence (Šebesta *et al*. 2003; Leonard *et a*l. 2004, 2005; Chong & Zegeye 2004).

Near isogenic lines of *Avena sativa*, conferring single major genes for resistance to *Puccinia coronata* var. *avenae*, have become the indispensable basis to carry out these analyses. The aim of the present study was to assess the physiologic specialization of oat crown rust populations in the Czech Republic during the period from 2004 to 2006, to find significant changes in virulence and to assess the resistance of 12 selected oat cultivars registered in the Czech Republic to oat crown rust.

MATERIALS AND METHODS

Samples of *Puccinia coronata* var. *avenae* originated from field trials of the Central Institute for Supervising and Testing in Agriculture at five localities: Lípa (Havlíčkův Brod district), Staňkov (Domažlice district), Krukanice (Plzeň-sever district), Hradec nad Svitavou (Svitavy district), Chrastava (Liberec district).

Susceptible oat cv. Neklan was inoculated with urediospores obtained from the leaves in greenhouse conditions as described by Klenová & Šebesta (2006). When the first symptoms of the disease developed, only leaves with a single rust pustule were further cultivated. Monopustule isolates obtained in this way were increased on cv. Neklan to the amount sufficient for further analyses.

Inoculation was carried out by means of a glass cyclone separator on the first fully expanded seedling leaf. After inoculation, the pots with plants were covered with glass cylinders to keep high air humidity and incubated at a temperature of 18°C for 2 days. After incubation the same greenhouse temperature was maintained and natural daylight was complemented by 16 h illumination with fluorescent tubes (Klenová & Šebesta 2006). The reaction types were evaluated in 10–14 days after inoculation, using the scale described by Stakman *et al*. (1962). Infection types from 0 to 2 indicated resistance while types 3 and 4 denoted susceptibility (Figure 1). The four-letter code of pathotype designation was used (Chong *et al*. 2000).

Physiologic specialization analyses were carried out on 23 differential oat lines (Table 1) in greenhouse condition. The basic set of 16 differentials required for pathotype designation according to the nomenclature proposed by Chong *et al*. (2000) was included.

Resistance to *Puccinia coronata* var. *avenae* was evaluated in 12 selected oat cultivars grown in the Czech Republic, i.e. Abel, Ardo, Atego, Auron, Azur, Dalimil, Izak, Avenuda, Neklan, Radius, Saul and Vok. Detailed characteristics of the registered varieties are available on the website of the Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic: http://odrudy.zeus.cz/ido/index.html?lang=en.

The cultivars were inoculated with 22 pathotypes (Table 2). The same inoculation and scoring method was used as described above.

RESULTS

The incidence of *Puccinia coronata* var. *avenae* was recorded in the Czech Republic during the period from 2004 to 2006. Neither serious epidemics nor zero occurrence of oat crown rust were observed. Crown rust infections were recorded between the last week of June and the first week of July in the monitored areas. Totally 79 monopustule isolates were obtained and evaluated.

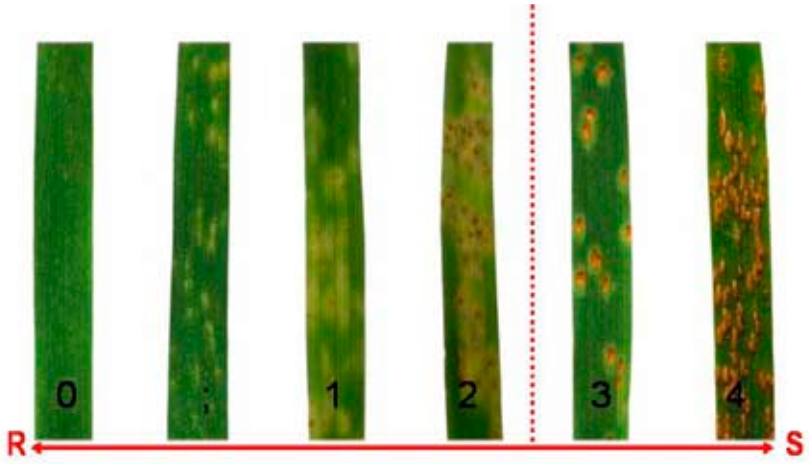

Figure 1. Scale for the assessment of oat crown rust in greenhouse tests

R – resistant types, S – susceptible types

Table 1. Frequencies of virulence to the differential lines of *Avena sativa* with single major genes for resistance to oat crown rust

Gene designation	Original source	Line	Virulence frequency (%)			
			2004	2005	2006	Average
Pc38	*A. sterilis CW491-4*	*Pendek × Pc38*	38.46	4.15	46.15	43.59
Pc39	*A. sterilis F-366*	*Pendek × Pc39*	0.00	0.00	0.00	0.00
Pc40	*A. sterilis F-83*	*Pendek × Pc40*	50.00	42.31	42.31	44.87
Pc45	*A. sterilis F-169*	*Pendek × Pc45*	42.31	23.08	23.08	29.49
Pc46	*A. sterilis F-290*	*Pendek × Pc46*	42.31	11.54	11.54	21.80
Pc48	*A. sterilis F-158*	*Pendek × Pc48*	0.00	3.85	3.85	2.57
Pc50	*A. sterilis Wahl No. 8*	*Pendek × Pc50*	0.00	0.00	0.00	0.00
Pc51	*A. sterilis CW-486*	*Iowa isolines X270 & X434*	23.08	3.85	3.85	10.26
Pc52	*A. sterilis Wahl No. 2*	*Iowa isoline X421*	0.00	0.00	0.00	0.00
Pc54	*A. sterilis CAV 1832*	*Pendek × Pc54*	23.08	7.69	7.69	12.82
Pc54 -1	*A. sterilis CAV 1833*	*Pendek × Pc54*	0.00	3.85	3.85	2.57
Pc55	*A. sterilis CAV 4963*	*Pendek × Pc55*	3.85	0.00	0.00	1.28
Pc56	*A. sterilis CAV 1964*	*Pendek × Pc56*	30.77	34.62	34.62	33.34
Pc58	*A. sterilis PI 295919*	*TAM-O-301*	7.69	0.00	0.00	2.56
Pc59	*A. sterilis PI 296244*	*TAM-O-312*	0.00	0.00	0.00	0.00
Pc60	*A. sterilis PI 287211*	*Coker 227*	3.85	3.85	3.85	3.85
Pc61	*A. sterilis PI 287211*	*Coker 234*	3.85	3.85	3.85	3.85
Pc62	*A. sterilis CAV 4274*	*Fraser Pc62*	0.00	0.00	0.00	0.00
Pc64	*A. sterilis CAV 4248*	*Makuru//Sun II Pc64*	34.62	30.77	30.77	32.05
Pc67	*A. sterilis CAV 4656*	*Makuru//Sun II Pc67*	11.54	34.62	34.62	26.30
Pc68	*A. sterilis CAV 4904*	*Makuru//Sun II Pc68*	0.00	0.00	0.00	0.00
Pc94	*A. strigosa* (*RL1697*)	–	0.00	3.85	3.85	2.57
Pc96	*A. sativa**	–	0.00	3.85	3.85	2.57

*the code unknown, the line was obtained from the Cereal Research Centre, Winnipeg

The number of virulence genes in the analyzed rust isolates varied from zero (none of the differentials was susceptible) to eight. Virulence frequencies to the differentials are shown in Table 1. No isolates virulent to the resistance genes *Pc39, Pc50, Pc52, Pc59, Pc62* and *Pc68* were observed and therefore these resistance genes can be considered as the most effective and recommended for resistance breeding. Contrarily, virulence to resistance genes *Pc38, Pc40, Pc45, Pc46, Pc56, Pc64* and *Pc67* occurred frequently.

High diversity of virulence patterns was detected (Table 3). The most common pathotypes were BLBG virulent to *Pc38* and *Pc56* (7 isolates), BBBC virulent only to *Pc64* (6 isolates), BBBB avirulent to all resistance genes in the used differentials (5 isolates), BLBG-67 virulent to *Pc38, Pc56* and *Pc67* (4 isolates), BLBB-67 virulent to *Pc37* and *Pc67* (4 isolates), LBBH virulent to *Pc40, Pc56* and *Pc64* (3 isolates), BLBB virulent only to *Pc38* (3 isolates) and BLBH virulent to *Pc38, Pc56* and *Pc64* (3 isolates). The following pathotypes were found twice: QLBC-67, QLBG-67, BLBD, LLBB, SBLB, BLBH and once were found the pathotypes SBBB, SLLM, SBLM, SBNL, SBNM, QLBH-67, GLBH, BLBC, QLBB, BLBH-96, LLBK-67, LBBB-67, NLBB, BLBF, LBLQ-54-1, QLBB-67, 94, LLBF-67, LLBH-96, LLBC, JLBK-67, LLBC-67, QBBB-67, QBBB, QDBC-60, 61, 67, SBLL-60, 61, 67, SBBC, LBBB, SBBB-55, SBBL, SBLC, SBBB, SBBB-67, SLBC, QBBC.

Pathotypes with unusual virulence patterns were also observed. The pathotype QDBC-60, 61, 67

Table 2. Reaction of 22 pathotypes on 12 registered oat cultivars

Cultivar	Virulent pathotypes
Vok	LBLQ-54-1; QDBC-60, 61, 67; SBBC; SLLM
Avenuda	BBBB; BLBD; BLBF; BLBG; BLBH; GLBH; LBBH; LLBB; LLBC; LLBF-67; LLBH-96; NLBB; QBBB; SBBC; SLLM
Dalimil	BBBB; BLBD; BLBF; BLBG; BLBH; GLBH; GLBH; LBBB; LBBH; LBBH; LLBC; LLBF-67; NLBB; QBBB; SBBC; SLLM; SLLM
Auron	BBBB; BLBD; BLBD; BLBF; BLBG; BLBH; BLBH; GLBH; GLBH; LBBH; LBLQ-54-1; LLBB; LLBC; NLBB; QBBB; QDBC-60,61,67; SBBC; SBBL; SLLM; SLLM
Isak	BBBB; BLBD; BLBD; BLBF; BLBG; BLBH; BLBH; GLBH; LBBB; LBBH; LBLQ-54-1; LLBB; LLBC; LLBF-67; LLBH-96; NLBB; QBBB; QDBC-60,61,67; SBBC; SLLM; SLLM
Abel Ardo Atego Azur Neklan Radius Saul	BBBB; BLBD; BLBD; BLBF; BLBG; BLBH; BLBH; GLBH; LBBB; LBBH; LBLQ-54-1; LLBB; LLBC; LLBF-67; LLBH-96; NLBB; QBBB; QDBC-60,61,67; SBBC; SBBL; SLLM; SLLM

was virulent to the resistance gene *Pc48,* pathotype SBNL virulent to *Pc58* or pathotype QLBB-67, 94 was virulent to resistance gene *Pc94*. These findings are important because the above-mentioned resistance genes were considered to be highly effective so far.

A low level of resistance of the tested oat cultivars to oat crown rust was recorded (Table 2). Only cv. Vok was susceptible just to 4 out of 22 applied pathotypes, other cultivars were found to be susceptible. The cultivars Abel, Ardo, Alegro, Azur, Neklan, Radius and Saul were susceptible to all pathotypes, whereas Avenuda to 16, Dalimil to 17, Auron to 20 and Isak to 21 pathotypes. The pedigree of the cv. Vok is [(Flämingssone × Ardo) × (Flämingssone × KR-81-1122)] × {(Flämingssone × Ardo) × [Flämingssone × (KR-81-1010 × Dragon)]}, sources of resistance are lines KR-81-1122 and KR-81-1010).

DISCUSSION

The high diversity in virulence patterns of the *Puccinia coronata* var. *avenae* population observed in our survey is not surprising; numerous oat crown rust studies repeatedly showed that many different virulence combinations occurred widely (Martens & Dyck 1989; Kolmer & Chong 1993; Chong & Zegeye 2004). The diversity of pathotypes can be caused by the sexual propagation of *Rhamnus cathartica*, an intermediate host of oat crown rust required for the completion of the sexual cycle, which is not rare either in the Czech Republic or in neighbouring countries. Asexual propagation may also contribute to oat crown rust diversity; mutations or somatic recombinations have already been proved (Zimmer & Schafer 1959; Zimmer *et al*. 1963; Bartoš *et al*. 1969). A limited selection pressure might also contribute to the high diversity of the pathotypes described in our survey, caused either by relatively small areas under oat or by cultivation of oat cultivars with very low resistance to *Puccinia coronata* var. a*venae* in the Czech Republic.

In the Czech Republic, the crown rust pathotypes with up to three different virulence genes were recorded most frequently. The dominance of pathotypes with a low number of virulence genes might be caused by prevailing cultivation of susceptible oat cultivars. Besides the pathotypes possessing a low number of virulence genes, we also observed highly virulent pathotypes possessing 6–8 virulence genes. These pathotypes may have been transported by wind from southern Europe. Isolates with similar virulence were obtained from Serbia and Monte Negro. The transport of rust urediospores is very often realised by wind from this distinct area (Šebesta *et al*. 2003).

Comparisons of our observations with previous records of the incidence and virulence of oat crown rust populations from the period 1995–2003 (Šebesta *et al*. 2003; Šebesta unpublished) are

Table 3. *Puccinia coronata* var. *avenae* pathotypes found in the Czech Republic (2004–2006)

Pathotype designation	Virulences (ineffective *Pc* genes)
SBBB	*Pc40, Pc45, Pc46*
BBBC	*Pc64*
BBBB	–
SLLM	*Pc40, Pc45, Pc46, Pc38, Pc51, Pc54, Pc64*
SBLM	*Pc40, Pc45, Pc46, Pc51, Pc54, Pc64*
SBNL	*Pc40, Pc45, Pc46, Pc51, Pc58, Pc54*
SBNM	*Pc40, Pc45, Pc46, Pc51, Pc58, Pc54, Pc64*
BLBG	*Pc38, Pc56*
LBBH	*Pc40, Pc56, Pc64*
BLBG-67	*Pc38, Pc56, Pc67*
BLBB	*Pc38*
QLBC-67	*Pc38, Pc45, Pc46, Pc64, Pc67*
QLBH-67	*Pc38, Pc40, Pc45, Pc56, Pc64, Pc67*
GLBH	*Pc40, Pc45*
BLBB-67	*Pc38, Pc67*
BLBC	*Pc38, Pc64*
QLBB	*Pc38, Pc45*
QLBG-67	*Pc38, Pc40, Pc56, Pc67*
BLBD	*Pc38, Pc55, Pc62*
BLBH-96	*Pc38, Pc56, Pc64, Pc96*
LLBK-67	*Pc38, Pc40, Pc56, Pc62, Pc64, Pc67*
LBBB-67	*Pc40, Pc67*
LLBB	*Pc38, Pc40*
NLBB	*Pc38, Pc40, Pc46*
BLBF	*Pc38, Pc62, Pc64*
LBLQ-54-1	*Pc40, Pc51, Pc54, Pc54-1, Pc56*
QLBB-67, 94	*Pc38, Pc 40, Pc45, Pc67, Pc94*
LLBF-67	*Pc38, Pc40, Pc62, Pc64, Pc67*
LLBH-96	*Pc38, Pc40, Pc56, Pc62, Pc64, Pc96*
LLBC	*Pc40, Pc38, Pc64*
JLBK-67	*Pc38, Pc45, Pc46, Pc56, Pc62, Pc64, Pc67*
BLBH	*Pc38, Pc56, Pc64*
SBLB	*Pc40, Pc45, Pc46, Pc54*
DBBK-67	*Pc46, Pc56, Pc62, Pc64, Pc67*
QBBB-67	*Pc40, Pc45, Pc67*
QBBB	*Pc40, Pc45*
QDBC-60,61,67	*Pc40, Pc45, Pc48, Pc60, Pc61, Pc64, Pc67*
SBLL-60,61,67	*Pc40, Pc45, Pc46, Pc51, Pc54, Pc60, Pc61, Pc67*
SBBC	*Pc40, Pc45, Pc46, Pc64*
LBBB	*Pc40*
SBBB-55	*Pc40, Pc45, Pc46, Pc55*
SBBL	*Pc40, Pc45, Pc46, Pc54*
SBLC	*Pc40, Pc45, Pc46, Pc51, Pc64*
SBBB	*Pc40, Pc45, Pc46*
SBBB-67	*Pc40, Pc45, Pc46, Pc67*
SLBC	*Pc40, Pc45, Pc46, Pc38, Pc64*
QBBC	*Pc40, Pc45, Pc64*

complicated because different sets of differential oat lines were used in both analyses. In our analysis, we used the nomenclature system by Chong *et al.* (2000). The set of eighteen differential oat lines coming from the European and Mediterranean Oat Disease Nursery (Šebesta 2000) was used in the Czech Republic by 2004. Compared to previous analyses, we included additional differential oat lines possessing the oat crown rust resistance genes *Pc40, Pc45, Pc46, Pc51, Pc52, Pc54, Pc94* and *Pc96* whereas oat lines possessing the oat crown rust resistance genes *Pc 54-2* and *Pc63* (members of EMODN) were not used. When we compared the virulence patterns of oat crown rust isolates sampled in 1995–2003 (Šebesta *et al.* 2003; Šebesta unpublished) with the results of virulence survey in our analysis we also found the resistance genes *Pc39, Pc50, Pc59, Pc62* and *Pc68* as highly effective so far; no occurrence of virulence to the mentioned resistance genes was recorded during the period from 1995 to 2006. However, the isolates virulent to resistance gene *Pc39* have already been recorded in Austria, Serbia or Israel and the isolates possessing virulence to *Pc68* were found in Austria (Šebesta *et al*. 2003). We have also recorded the virulence to resistance gene *Pc50* in samples from Serbia and Israel in 2004 and from Hungary in 2006.

An increase in virulence to resistance genes *Pc38* and *Pc64* was observed. Whereas only individual isolates virulent to *Pc38* and *Pc64* were recorded between 1995 and 2003 (Šebesta *et al*. 2003; Šebesta unpublished), virulence to these resistance genes was common in our survey. We also found two single isolates virulent to resistance gene *Pc55*, which is the first finding in the Czech Republic for the time being. Virulences to resistance genes *Pc48, Pc54-1, Pc55, Pc58, Pc60, Pc61, Pc94* and *Pc96* were observed only scarcely in our analyses as well as in earlier research (Šebesta *et al*. 2003). These genes, as well as genes *Pc39, Pc50, Pc52, Pc59, Pc62* and *Pc68*, mentioned earlier, are the most promising sources of resistance to crown rust for resistance breeding in the Czech Republic. Cultivars registered in the Czech Republic possess medium resistance to crown rust on oat. Only the cultivar Vok was classified in the field trials of the Central Institute for Supervision and Testing in Agriculture as highly resistant. This corresponds with our results on the resistance of the cultivar Vok in a greenhouse.

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