Analysis of Populations of *Pyrenophora teres* on Barley in the Czech Republic*

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Abstract

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One of the diseases that have become important in the Czech Republic recently is net blotch of barley caused by *Pyrenophora teres* (Died.) Drechs., with the imperfect state *Drechslera teres*. In 1995-1997 infected leaves of both spring and winter barley were collected in various stands and climatic regions. Almost 400 isolates of the pathogen were obtained and tested for virulence using a differential set (CI 5791, CI 2750, CI 9819, C 8755, Steudelli, Harbin, C 29192, CI 739, Tifang, and the susceptible control Beate). To assess their reaction, the laboratory method for testing leaf segments on benzimidazole was used. The most stable resistant responses, compared also with previous tests from 1991–1994, were found in CI 739 and Tifang where the frequency of virulent isolates did not exceed 10% of all tested ones. These genotypes should be involved in practical breeding of barley for resistance to the pathogen.

Key words: *Hordeum vulgare*; net blotch; leaf segment test

Barley is an economically important commodity. Similar to other crops, efforts are being made to release resistant cultivars to growers that would require no or minimal chemical treatments. The fungal diseases that had to be controlled during the last years are net blotch, caused by the facultative pathogen *Pyrenophora teres* (Died.) Drechs., and leaf blotch caused by *Rhynchosporium secalis* (Oud.) Davis. Under favourable conditions, both diseases may become a serious threat to both grain yield and malt quality. Especially severe epidemics of net blotch developed at many sites of the Czech Republic during 1996 and 1997. Winter barley was infected at a higher level by a spot form of the pathogen, *Pyrenophora teres* f. *maculata*, while spring barley was more sensitive to the classical net form, *Pyrenophora teres* f. *teres*. The pathogen may cause considerable yield losses ranging from 10 to 40% according to STEFFENSON & WEBSTER (1992). That is in accordance, for instance, with results of ARABI *et al.* (1992) who assessed a yield loss of 20% in the untreated control in his experiments with chemical products. Similar data were reported for the Czech Republic by VÁŇOVÁ (1996) with the cultivar Forum that is completely resistant to powdery mildew. In an experiment on the effect of the fungicide Tilt (a.i., propiconazole), the untreated control had a mean infection by net blotch of the flag and two upper leaves of 33.7%, while the treated variant had a mean infection of 20.2% and showed a significant yield increase of 2 t/ha, i.e., 34.6%.

Breeding for resistance to this pathogen is difficult because of its high intraspecific variability. The physiological specialization of the fungus was described as early as in the 1940s. Some studies have been focused on a differential set for the pathogen (AFANASENKO & LEVITIN 1979; GACEK 1985; TEKAUZ 1990; AFANASENKO *et al.* 1995). Pathogen populations were analyzed in the Czech Republic in 1991–1992, when 72 pathotypes were identified on 15 cultivars using isolates from six locations (MINAŘÍKOVÁ 1993). In the period from 1993 to 1994, 46 pathotypes were distinguished on 21 cultivars using 56 isolates from the location Mohelnice (MINAŘÍKOVÁ 1995).

The pathogen occurs in two forms that were described by SMEDEGARD-PETERSEN in 1971. They are the spot and classical net form, and have been reported in various regions of the world (e.g., BRANDL & HOFMANN 1991; ARABI *et al.* 1992; TEKAUZ 1990, and others). In 1995, the causal agent of the spot type, *Drechslera teres* f. *maculata,* was isolated in the Czech Republic on the winter barley cultivar Kromoz for the first time. The spot form occurred on winter barleys far more frequently than the net form (MINAŘÍKOVÁ 1996).

Analyses of local pathogen populations were carried out prior to selection of suitable donors for breeding pro-

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grammes aimed at the development of genotypes resistant to the pathogen. It is a challenging and long-term process because effective donors are mostly wild materials from original centres of the cultivation of barley. They are multirowed, low-yielding, lodging, and susceptible to other leaf diseases, particularly to powdery mildew (Tifang, Harbin, CI 739, CI 2750, C 29192, Steudelli, and others), and therefore need multiple backcrosses. Highly resistant materials are those which show susceptibility to maximal 10% of the isolates of the pathogen population. Thus, the possible donors are tested for resistance to local populations, i.e. the populations from various locations in different agroclimatic regions.

MATERIALS AND METHODS

Responses of donors to populations from different or individual locations are usually very variable, but great variability can also appear within a single population. An effective method to assess the reaction of donors is the laboratory method that tests leaf segments on a benzimidazole solution. It allows us to determine the percentage of isolates virulent to a genotype. Effective donors are genotypes with a broad effectiveness against different populations, that means the lowest number of isolates are virulent on them.

The infected material was collected during the growing season on currently grown barley cultivars and in different climatic regions. Diseased leaves were stored in paper bags at room temperature. Necrotic parts of leaf blades were excised, surface-sterilized using copper sulfate pentahydrate (up to 15 min) and rinsed with distilled water (for 5 min). The leaf segments were placed on potato-lactose agar (PLA) in Petri dishes under continuous fluorescent light. After 4–5 days, single-spore isolations were made and individual isolates incubated on PLA at 20–23°C. The inoculum was mostly prepared from one dish. Conidia were scraped off the surface of 10-day old cultures, mixed in distilled water and filtered through twofold mull. A concentration of 5 000 to 10 000 conidia per ml was measured with a micro-pipette. Tween 80 wetting agent was added to the inoculum.

To assess the response of each genotype of the host to each isolate of the pathogen, the laboratory method for testing leaf segments on a benzimidazole solution was applied. The procedure has been described by AFANASENKO *et al.* (1995) in greater detail. Segments 15 mm long of healthy primary leaves were placed on filter paper soaked with 0.004% benzimidazole solution. Drops of the inoculum were applied to the surface of segments. Plastic dishes with segments were left in diffused light for 24 h and then transferred under continuous fluorescent light. Each isolate was tested on each genotype of the host in four replicates. Six days later, responses were scored using the 0–4 scale where a lower value indicates higher resistance. A mean of the four replications up to 2.0 was considered as resistant response, a mean higher than 2.1 as susceptible. Responses of the genotypes were evaluated separately for individual locations and, at the same time, for all populations from the locations. The aggressiveness of isolates from the locations was compared.

RESULTS

1995

In 1995 (Table 1), 196 isolates from 10 locations (Staré Místo, Velký Beranov, Červenka, Stupice, Malé Hradisko, Rýmařov, Strukov, Kroměříž, Postoupky, and Hradec nad Svitavou) were tested. Overall, the lowest number of isolates was virulent to Tifang (8%), but at the location Velký

Table 1. Frequency of virulent isolates in populations from 10 locations of the Czech Republic on differential cultivars of spring barley (1995)

	Frequency of virulent isolates [%] out of the total number of isolates per location										
Cultivar	Staré Místo 11*	Velký Beranov 15*	Červenka 17*	Stupice 39*	Malé Hradisko 4*	Rýmařov 19*	Strukov 3*	Kroměříž 24*	Postoupky 13*	Hradec 41*	mean
CI 5791	0	13	0	13	71	37	67	4	54	34	25
CI 2750	18	53	0	10	36	0	0	54	15	0	17
CI 9819	9	0	47	26	0	37	33	4	46	22	22
C 8755	0	7	71	51	36	26	100	25	54	46	40
Steudelli	9	13	6	13	14	11	33	4	23	12	12
Harbin	0	13	0	8	43	0	0	17	15	10	11
C 29192	36	27	12	18	7	0	33	30	23	7	17
CI 739	0	33	0	5	14	0	0	17	31	7	10
Tifang	0	27	0	15	21	5	0	4	8	0	8
Beate	64	100	100	95	86	47	100	79	69	81	82
Location mean	14	29	24	25	33	17	37	24	34	22	

*number of tested isolates at the location

Beranov it was 27%. Further, 10% of all isolates were virulent on CI 739, but 33% of the isolates from Velký Beranov were virulent on it. However, the aggressiveness of the population from this location was not the highest of the tested populations. The third lowest number of virulent isolates was found for Harbin (11%), with the exception of the population from Malé Hradisko where almost 43% of the isolates were virulent. Cultivar Steudelli with 12% of virulent isolates ranked fourth within resistant cultivars. By contrast, 22% of the isolates were virulent to CI 9819, which was completely resistant to the populations from Velký Beranov and Malé Hradisko. It will thus be useful to combine the resistance of this entry with those from CI 739 and Harbin. Responses varied considerably in CI 5791, with a frequency of virulent isolates from 0 to 71%; likewise C 8755 showed a response range from 0 to 100%. A maximum frequency of isolates virulent on the susceptible control cultivar Beate, i.e., 100%, was assessed at three locations (Velký Beranov, Červenka and Strukov), but only 47% of the isolates from Rýmařov were virulent on it. The spot form of the pathogen was detected only at Staré Místo; the aggressiveness of this population was the lowest of all tested locations.

1996

In 1996 (Table 2), 88 isolates from populations of the locations Štěpánkovice, Velký Beranov, Lužany, Kroměříž, Kujavy and Chrlice were investigated. An identical percentage of virulent isolates was recorded for CI 9819 and CI 739, while isolates virulent to CI 9819 occurred in one population only (Chrlice). The same number of virulent isolates (11%) was also assessed for CI 2750, Steudelli and C 29192. There were 14% of the isolates virulent to cultivar Tifang, which was the highest percentage of the 3 years. At four locations, 100% of the isolates were virulent to the susceptible control Beate. The spot form of the pathogen was found at Lužany and Kujavy. The population from the location Kujavy was least aggressive, which corresponds with results obtained in 1995. However, the population from Lužany was the most aggressive one from all locations.

1997

A total of 115 isolates (Table 3) from six locations (Kroměříž, Zářičí, Postoupky, Stupice, Lužany, and Hradec nad Svitavou) were tested. The first three locations are rather close to each other, but populations were sampled at different growth stages as follows: the population from Postoupky was collected in early spring (on the cultivar Beate at tillering), the populations from Kroměříž and Zářičí were also collected on Beate in late June (GS 69) and mid-October, respectively. The aggressiveness of these populations corresponds with dates of collection: the population collected earliest was least aggressive. In general, the lowest number of virulent isolates was from Lužany, where the population consisted of the spot form of the pathogen. The lowest number of virulent isolates was found for Tifang (1%) and CI 739 (3%). Isolates virulent to Tifang were detected at one location only (Postoupky), and those virulent to CI 739 at two locations (Postoupky and Zářičí). There were 8% of isolates virulent to both CI 2750 and Harbin. Low percentages of virulent isolates were observed also for Steudelli (10%), C 29129 (13%) and CI 9819 (14%). The control cultivar Beate was susceptible to a maximum number of isolates at three locations, and was susceptible to the highest number of virulent isolates of the three years studied.

DISCUSSION

We can compare these results (Table 4, Fig. 1) with analyses which were carried out in 1991–1992 (100 isolates

Table 2. Frequency of virulent isolates in populations from six locations of the Czech Republic on differential cultivars of spring barley (1996)

	Frequency of virulent isolates [%] to a total number of isolates per location						
Cultivar	Štěpánkovice 10*	Velký Beranov 11*	Lužany 9*	Kujavy 25*	Chrlice 19*	Kroměříž 14*	mean
CI 5791	0	0	0	36	36	0	18
CI 2750	0	27	44	8	5	0	11
CI 9819	0	0	0	0	21	0	5
C 8755	50	91	56	4	47	43	41
Steudelli	20	46	22	4	0	0	11
Harbin	0	27	55	3	10	7	16
C 29192	0	0	22	0	42	0	11
CI 739	0	0	22	0	10	0	5
Tifang	20	36	44	8	0	0	14
Beate	100	100	100	68	100	92	90
Location mean	19	33	37	13	27	14	

*number of tested isolates at the location

Table 3. Frequency of virulent isolates in populations from six locations of the Czech Republic on differential cultivars of spring barley (1997)

Cultivar	Frequency of virulent isolates [%] to a total number of isolates per location						
	Kroměříž 29*	Zářičí 17*	Postoupky 29*	Stupice 14*	Lužany 12*	Hradec 14*	mean
CI 5791	32	71	39	43	25	7	36
CI 2750	0	24	0	14	8	0	8
CI 9819	28	12	14	21	8	0	14
C 8755	88	77	32	57	50	78	63
Steudelli	11	0	35	7	8	0	10
Harbin	0	12	14	14	8	0	8
C 29192	35	12	7	1	0	21	13
CI 739	0	6	11	0	0	0	3
Tifang	0	0	7	0	0	0	1
Beate	98	100	84	100	83	100	94
Location mean	29	31	24	26	19	21	

*total number of tested isolates at the location

from six locations) and in the following years (245 isolates from ten locations). Seventy percent of the isolates were virulent to the susceptible control Beate in 1991–1992, 89% in the following years, 82% in 1995, 90% in 1996, and 94% in 1997. Only rarely was the frequency of isolates virulent to individual genotypes nearly the same (e.g., the responses of CI 739 in 1993–1994 and 1996, of Steudelli in 1995 and 1996). This may be caused by several factors, for instance by the fact that the populations from the same locations are not tested each year. However, the most important factor is that the race spectrum of the pathogen is still changing.

At some locations samples infected with the spot form were collected. The population from Lužany (where infected material was collected on spring barley in early spring of 1996) tended to produce sterile spots, i.e., no conidia developed even on nutritive medium, so that 110 samples gave only 12 isolates. This is explained by SMEDEGARD-PETERSEN (1971) who suggests the possibility that species of the genus *Pyrenophora* hybridize with each other and part of the progeny produces sterile spots. Isolates from Kujavy gave results similar to those from Velký Beranov, i.e., many pure cultures did not produce a sufficient concentration of inoculum even under standard incubation conditions.

Table 4. Frequency of virulent isolates in populations of the pathogen in the Czech Republic (1991–1997)

Cultivar	Frequency of virulent isolates [%]					
	100	245	196	88	115	744* /mean
CI 739	7	4	10	5	3	6
Tifang	12	6	8	13	1	8
CI 5791	48	41	25	18	36	34
CI 2750	18	14	17	11	8	14
CI 9819	16	26	22	5	14	17
C 8755	–	–	40	41	63	48
Steudelli	30	23	12	11	10	17
Harbin	28	1	11	16	8	15
C 29192	–	28	17	11	13	17
Beate	70	89	82	90	94	85
Testing period	1991–92	1993–94	1995	1996	1997	

*total number of tested isolates at the location

Besides studying the epidemiology of the pathogen, the aim of population analyses is to detect suitable donors for the programme to breed for resistance to it. Donors exhibiting wide effectiveness to many different pathogen populations will be used as parents. This effectiveness is understood in both spatial and temporal terms. Despite the fact that highly resistant materials are genotypes on which 10% or fewer of the isolates are virulent, we cannot exclude materials exceeding this limit from the list of suitable donors. It is necessary to study their long-term reactions and the trend during several years, and compare them with results obtained by other authors. The most promising donors among the differentials proved to be cultivars Tifang and CI 739, which repeatedly exhibited very high resistance. GACEK (1985) reports 72% of isolates virulent for genotype CI 739 in Poland. It is not unusual that a given genotype "fails" at a certain location or in a certain year. If such responses appear again, it is necessary to combine the genotype with another donor for which a low number of virulent isolates occurred at this location. This is the case for CI 739 and Tifang at Velký Beranov, where a higher percentage of isolates virulent to these genotypes occurred. Therefore, they were combined with CI 9819 in the subsequent hybridization programme. This genotype showed to be an effective donor

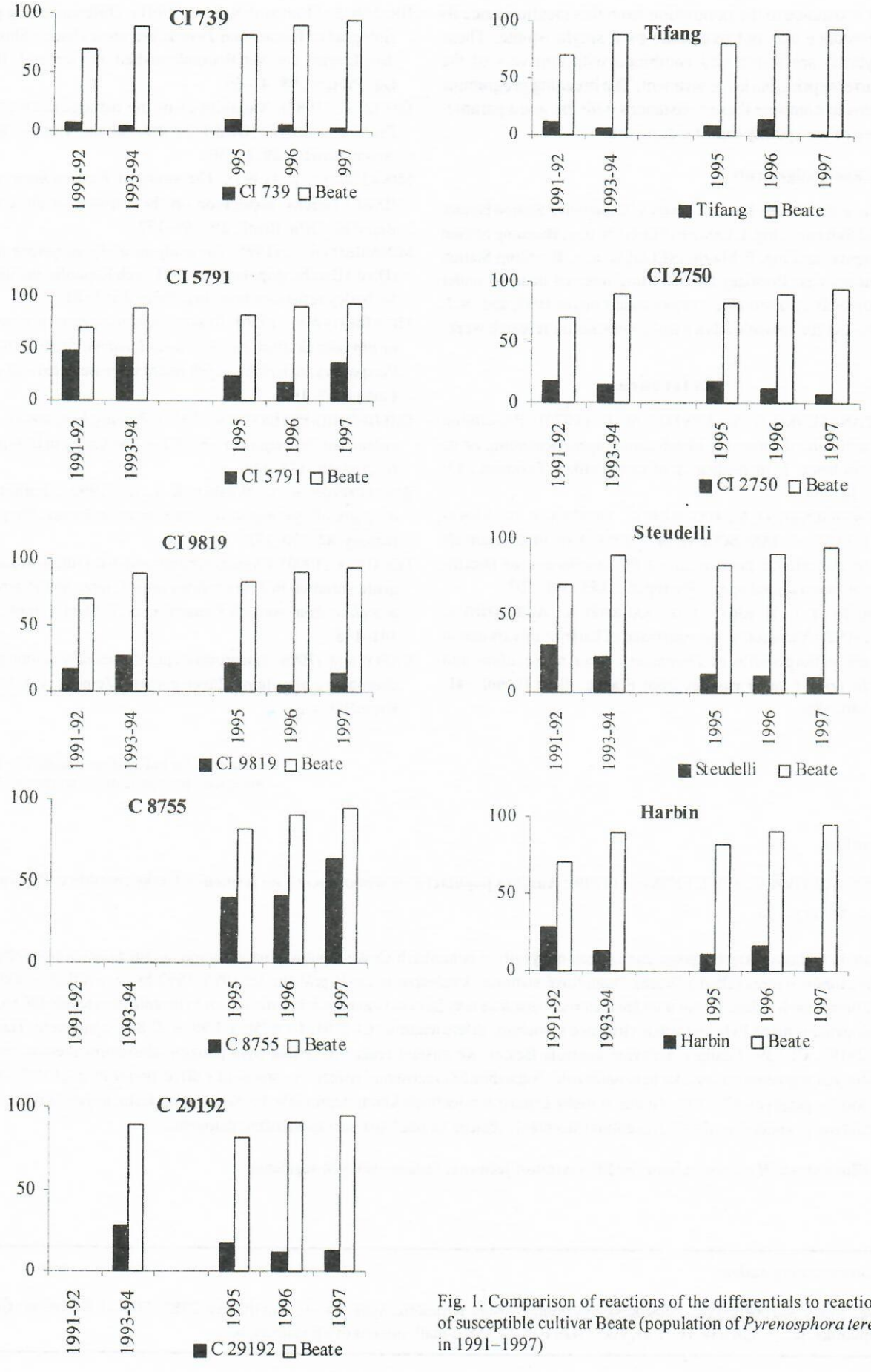

Fig. 1. Comparison of reactions of the differentials to reaction of susceptible cultivar Beate (population of *Pyrenosphora teres* in 1991–1997)

of resistance to the population from this location since its resistance was not overcome by a single isolate. These hybrids are tested and combined with cultivars of the current spring barley assortment. The breeding programme aims to combine these resistances with the good parameters of our quality malting cultivars.

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Souhrn

MINAŘÍKOVÁ V., POLIŠENSKÁ I. (1999): **Analýza populací** *Pyrenophora teres* **na ječmeni v České republice.** Pl. Protect. Sci., **35**: 115–120.

Jednou z chorob, které v posledních letech nabývají v podmínkách České republiky na významu, je hnědá skvrnitost ječmene *Pyrenophora teres* (Died.) Drechs., konidiové stadium *Drechslera teres*. V průběhu let 1995–1997 byl z rozdílných půdních a klimatických oblastí sbírán infekční materiál, napadené listy jarního i ozimého ječmene. Z nich bylo izolováno téměř 400 izolátů patogena, u nichž byla testována virulence k souboru diferenciátorů (CI 5791, CI 2750, CI 9819, C 8755, Steudelli, Harbin, C 29192, CI 739, Tifang a náchylná kontrola Beate). Ke zjištění reakce materiálů byla použita laboratorní metoda testace listových segmentů na roztoku benzimidazolu. Nejstabilnější rezistentní reakce i ve srovnání s dřívějšími testy z let 1991–1994 vykázaly genotypy CI 739 a Tifang, u nichž četnost virulentních klonů nepřesáhla 10 % ze všech testovaných izolátů. Tyto genotypy je vhodné využít v rezistentním šlechtění ječmene na odolnost vůči uvedenému patogenu.

Klíčová slova: *Hordeum vulgare*; hnědá skvrnitost ječmene; testace listových segmentů

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