

Pathogenic Variation in Some Isolates of *Pyrenophora teres* f. sp. *maculata* on Barley

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ABSTRACT

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Pathogenic variation in *Pyrenophora teres* f. sp. *maculata*, causal organism of Pyrenophora spot blotch of barley (*Hordeum vulgare*), was evaluated using 20 barley cultivars and 14 fungus isolates from Montana, Morocco, Tunisia, and Turkey. All isolates were avirulent on Unitan, CI 5401, CI 9214, CI 9440, and CI 9776 and virulent on Nupana, Klages, Dekap, Clark, and CI 13727. Tifang, CI 7584, CI 9819, and CI 9825 showed differential reactions between isolates from Montana and those from countries other than the United States. Arimont, Galt, and Steptoe showed differential reactions to both Montana and Mediterranean isolates. A cluster analysis indicated a mean disease rating of 4.3 as the separation point between avirulent and virulent reactions. The frequency of virulence was highest for isolate Turk 74-Pt6 and lowest for Mor 82-1.

Additional key words: net-spot blotch

Three well-known *Helminthosporium* disease complexes of barley are spot blotch, caused by *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dast., teleomorph of *Bipolaris sorokiniana* (Sacc.) Shoem. (syn. *Helminthosporium sativum* PKB); *Pyrenophora graminea* (Drechs.), teleomorph of *Drechslera graminea* (Rabenh. ex Schlecht.) Shoem. (syn. *H. gramineum* Rabh.); and *P. teres*, teleomorph of *D. teres* (Sacc.) Shoem. (syn. *H. teres* Sacc.). Recently, however, one more *Helminthosporium* sp. causing spot symptoms similar to those caused by *C. sativus* (5) and morphologically similar to *P. teres* (11) has been reported occasionally in epidemic proportions from Australia (6), Canada (14), Denmark (11), Finland (8), Norway (5), Morocco, Tunisia, Turkey, and the United States (1).

The first spot symptoms of this disease were observed by McDonald (9) in Canada, but Smedegard-Petersen (11) first demonstrated that the spot-producing pathogen differed from the net blotch pathogen only in symptom

production on barley and designated the disease it caused as net-spot blotch. Consequently, he described two new forms, *P. teres* Drechs. f. sp. *teres* Smedeg. (*P. t. f. sp. teres*) (net blotch) and *P. teres* Drechs. f. sp. *maculata* Smedeg. (*P. t. f. sp. maculata*) (net-spot blotch).

In 1981, severe spot symptoms observed on many barley cultivars at Fairfield, MT, were indistinguishable from spot blotch, but conidia of the pathogen corresponded to *P. teres*. After isolation and inoculation, similar spots formed on barley seedlings and adult plants. The estimated loss of barley grain yield at Fairfield exceeded 10% when comparisons were made with long-term yield averages in the absence of the disease.

The symptoms produced by *P. t. f. sp. maculata* on barley leaves were spots of various shapes and sizes in contrast to typical dark brown longitudinal and transverse netlike necrotic streaks caused by the net blotch pathogen. Smedegard-Petersen (11) observed distinct dark brown lesions 3–6 mm long surrounded by a chlorotic zone of varying widths on the leaf blade. Later, different types of symptoms such as dark brown necrotic lesions, chlorosis, water-soaking of affected tissues, and general necrosis and blighting were described (11). Makela (8) observed two types of dark brown spots on barley in Finland: large spots measuring 0.5 × 0.5–3.0 mm and smaller spots measuring 0.5 × 0.5–2.00 mm. Elliptical, fusiform, or irregularly shaped necrotic lesions were the common symptoms observed with this pathogen in Canada (14).

The common name "net-spot blotch" designated for the new disease by Smedegard-Petersen (11) may be somewhat misleading to both growers and

research scientists. Therefore, a new common name, "Pyrenophora spot blotch" (PSB), for the spot disease caused by *P. t. f. sp. maculata* is proposed and is used throughout this paper. Smedegard-Petersen (11) tested 12 barley cultivars resistant to North American *P. teres* isolates (2,10) and observed different reactions to net blotch (PNB) and PSB isolates in Denmark (11). Cultivars usually gave an intermediate reaction to PSB. On the basis of lesion characteristics and extent of chlorosis and necrosis, PSB symptoms have usually been considered less accentuated than those caused by PNB (11,13). Resistance to PSB was often not associated with resistance to PNB (6). Differences in disease reactions were also observed among isolates of PSB from different countries. Khan and Tekauz (6) noted that disease reactions with Australian isolates were different from those from Canada on some barley cultivars. Bockelman et al (1) observed that isolates from Morocco, Tunisia, and Turkey generally induced bigger individual lesions and different reactions than those from Montana. The objectives of this study were to extend information on distribution and pathogenic variation among different isolates of *P. t. f. sp. maculata* (PSB).

MATERIALS AND METHODS

Isolate selection and designation. Over several years, more than 100 isolations were made from barley leaves showing spot-type symptoms both in Montana and the Mediterranean areas. From the isolations positive for PSB, selections were made for further evaluation. In preliminary studies on virulence groups, a total of 142 barley cultivars from the United States, Ethiopia, and Nepal were inoculated with the PSB isolates and evaluated for host-parasite reaction. Emphasis was placed on selecting isolates showing differential reactions on specific cultivars investigated in this study or previous work and on sampling of several Montana and Mediterranean locations. Subsequently, 14 isolates of PSB were selected for evaluation on 20 barley cultivars. Samples from different cultivars within a collection area were similar in virulence and were thus bulked for later evaluations. The PSB isolates were initially evaluated on barley cultivars Dekap, Klages, CI 5791, and Unitan and later increased in isolation on Dekap—an apparent "universal suscept." Isolates were named on the basis of state or country and year of collection.

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Isolate maintenance and pathogen isolation. The isolates chosen for study are listed in Table 1. Leaf tissue showing PSB symptoms was maintained at about 5 C for up to 1 yr, then cut into pieces 10 mm long and surface-sterilized in 1% sodium hypochlorite solution (NaOHC1) for about 2 min. Three or four leaf pieces of each sample were transferred to petri plates containing 2% water agar and incubated at 21 C with alternating 8 hr of fluorescent light (6.2W/m²) and 16 hr of darkness. Some isolates sporulated profusely after 24 hr; others required up to 72 hr for adequate sporulation. Conidia emerging from the leaf tissue were transferred singly onto the centers of petri plates containing V-8 juice agar (V-8 juice, 170 ml; Bacto agar, 20 g; calcium carbonate, 3 g; and distilled water, 830 ml). These plates, each containing a single conidium, were incubated for 15–20 days under the same conditions as described before. Monoconidial cultures up to 2 mo old maintained on V-8 agar were used for further subculturing. Subculturing was done once by transferring masses of mycelia containing conidiophores and conidia onto fresh agar. Subculturing more than once reduced conidial production. The subcultures were incubated for 10–11 days before use for inoculations.

Inoculum preparation. Inoculum was prepared by adding 50 ml of double-distilled water to each culture and scraping off mycelia, conidiophores, and conidia with a glass microscope slide or rubber-tipped rod. The conidial suspension was stirred with a magnetic stirrer for 4–5 min to separate conidia from conidiophores and mycelia, then filtered through several layers of gauze. About 0.1 ml of Tween 20 (polyoxyethylene sorbitan monolaurate) was added per 100 ml of the inoculum suspension. The conidial concentration was standardized at 3.2×10^4 /ml (1,14). Preliminary tests showed this concentration adequate for uniform infection.

Cultivar selection and planting. On the basis of earlier tests, 20 barley cultivars (Table 2) were chosen for evaluation of the PSB isolates. Plantings were made in Bozeman clay loam soil mixed with sand at a ratio of four parts clay loam to one part sand. About 12 seeds each of 10 cultivars were planted in a plastic flat (18 × 18 × 5 cm). Because of space limitations, studies were performed using 10 cultivars and five isolates each in six groups. Preinoculation and postinoculation incubation was at 21 C with 12 hr of light (3.9×10^4 erg/cm²/sec) and 13 C with 12 hr of darkness. Each treatment combination was replicated four times with a completely randomized design.

Inoculation. Ten-day-old barley seedlings were inoculated on a turntable by spraying 60 ml of the inoculum suspension with an atomizer driven by compressed air. Inoculated plants were incubated in a saturated dark dew chamber maintained at 21 ± 2 C for 24 hr, then returned to the growth chambers.

Disease rating. Disease ratings were made 10 days after inoculation on a scale of 1–10, where 1 = minute pinpoint or fleck-type lesions, without visible chlorosis and necrosis on the leaf blade; 2 = minute pinpoint or small (1–2 mm long) necrotic lesions, slight chlorosis on the leaf blade, and less than 20% leaf tip necrosis; 3 = small to large (1–2 mm or larger) necrotic lesions, slight chlorosis on the leaf blade, and 21–30% leaf tip necrosis; 4 = small to large necrotic lesions, slight to moderate chlorosis on the leaf blade, and about 31–40% leaf tip necrosis; 5 = small to large necrotic lesions, slight to moderate chlorosis on the leaf blade, and about 41–50% leaf tip necrosis; 6 = coalescing lesions, moderate chlorosis on the leaf blade, and 51–60% leaf necrosis; 7 = coalescing lesions, moderate to severe chlorosis, and 61–70% leaf necrosis; 8 = coalescing lesions, severe chlorosis, and 71–80% leaf necrosis; 9 = coalescing lesions, more necrosis than chlorosis, and 81–90% leaf

necrosis; and 10 = coalescing lesions, more necrosis than chlorosis, and less than 10% green area visible on the leaf blade.

A cluster analysis using BMDP (Biomedical software packages) (3) considered means of the disease ratings of four replicates to distinguish and separate resistant and susceptible reactions of barley that had been rated by the scale of 1–10. A separation point for resistant and susceptible classes was calculated by taking the average disease ratings of the two middle clusters and adding the standard error of the means (4). The average disease rating of the two middle clusters was calculated as 3.85. A standard error of the mean was calculated by taking the square root of the error mean square and dividing it by the square root of replicates ($SE = \text{error MS}/r = 0.80/4 = 0.45$). Because few cultivars with intermediate disease reactions were included in the experiment, the final separation point was calculated by adding the standard error to the average value instead of subtracting. The final separation point was then $3.85 + 0.45 = 4.30$ (4). Cultivars rated higher than 4.3 were considered susceptible and others were considered resistant. The frequency of virulence of each isolate was calculated by dividing the number of cultivars with a susceptible reaction (mean disease rating greater than 4.3) by the total number of 20 cultivars.

RESULTS

Of more than 100 isolations made from barley showing leaf spot symptoms, many did not result in cultures of PSB. Only nine samples from Montana were positive for PSB. All samples from Nepal were caused by *Cochliobolus sativus*; those from Syria were caused by either *C. sativus* or *P. t. f. sp. teres*. Some spots were also caused by genetic necrosis.

Table 1. Investigated isolates of *Pyrenophora teres* f. sp. *maculata* from different geographic regions

Isolate designation	State or country of origin	Place of collection	Year of collection
Mt FF81E ₁ ^a	Montana	Fairfield	1981
Mt FF82-12	Montana	Fairfield	1982
Mt Sun82-2	Montana	Sun River	1982
Mt Conrd82	Montana	Conrad	1982
Mt Lewis82-7	Montana	Lewistown	1982
Mt Eddy82-5	Montana	Eddy's Corner	1982
Mt Plent82	Montana	Plentywood	1982
Mt Lav82	Montana	Lavina	1982
Mt Fromb82	Montana	Fromberg	1982
Mor 82-1	Morocco	Rabat	1982
Tun 75 ^a	Tunisia	Tunis	1975
Tun 79-30 ^a	Tunisia	Tunis	1979
Tun 79-38 ^a	Tunisia	...	1979
Turk 74-Pt6 ^a	Turkey	...	1974

^a Isolates previously described.

Table 2. Barley cultivars used in determining pathogenic variation in isolates of *Pyrenophora teres* f. sp. *maculata*

Cultivar	CI number	Row type
Dekap	3351	2
Klages	15478	2
Nupana	16559	2
Steptoe	15229	6
Clark	15857	2
Tifang	4407-1	6
Galt	11770	6
Unitan	10421	6
Arimont	15509	6
	5401	6
	5791	2
	5845	6
	7584	6
	9214	6
	9440	6
	9699	6
	9776	6
	9819	2
	9825	2
	13727	2

Several isolates from Morocco, Tunisia, and Turkey were confirmed as PSB. Of 142 barley cultivars inoculated in preliminary studies with the various isolates of PSB, 48 were resistant to all isolates. Logistics and other considerations (given under Materials and Methods) resulted in the selection of 14 PSB isolates and 20 barley cultivars for further study.

Small water-soaked areas were observed on the leaf blades 24 hr after inoculation, and visible necrotic lesions appeared 3–4 days later. The lesions initially were grayish or brownish and consisted of circular or elliptical areas less than 1 mm in diameter. Initial lesions induced by PSB isolates from Montana varied from pinpoint to 1–2 mm in diameter and 1–6 mm long; lesions produced by isolates from Morocco, Tunisia, and Turkey were generally larger (1–4 mm wide × 2–8 mm long).

Lesions were usually surrounded by variable chlorosis 4–5 days after inoculation, depending on the cultivar and isolate. Isolates from Montana produced more chlorosis and necrosis than those from the Mediterranean area. In susceptible cultivars, the necrotic and chlorotic areas generally developed at the tip of the leaf blade and extended down to the leaf sheath. Preliminary studies showed that resistant and susceptible cultivars produced similar lesion numbers and that increased sporulation was directly associated with increased chlorosis and necrosis. The isolates from Montana produced the largest lesions on

CI 5791, Clark, Dekap, and Klages.

By cluster analysis, the cultivars were clustered into six groups or classes (Table 3), then designated highly resistant, resistant, moderately resistant, moderately susceptible, susceptible, and very susceptible. Considering any disease rating higher than 4.3 indicated susceptibility or virulence, an analysis of variance (7) showed highly significant differences among the cultivars and isolates (Table 4). Montana isolates were virulent on barley cultivars Nupana, Klages, Dekap, Clark, and CI 13727, whereas Mediterranean isolates were virulent on Nupana, Klages, Dekap, Clark, CI 13727, CI 7584, and CI 9819. Montana isolates were avirulent on Unitan, CI 5401, CI 9214, CI 9440, CI 9776, CI 7584, CI 9819, CI 9825, and Tifang, whereas Mediterranean isolates were avirulent on Unitan, CI 5401, CI 9214, CI 9440, and CI 9776. Differential virulence was noted for Montana isolates on barley cultivars Arimont, Galt, Steptoe, and CI 5791 and for Mediterranean isolates on Arimont, Galt, Steptoe, CI 5845, CI 9699, CI 9825, and Tifang (Table 5).

The virulence frequency of all PSB isolates studied is shown in Table 6. Among Mediterranean isolates, Turk 74-Pt6 had the highest percentage of virulence at 75% and isolate MOR 82-1 had the lowest at 35%. Within the Montana isolates, MT Plent 82 had the highest percentage of virulence at 60% and isolate MT Sun 82-2 had the lowest at 35%.

Table 3. Cluster analysis of mean disease ratings of 14 isolates of *Pyrenophora teres* f. sp. *maculata* on 20 barley cultivars

Cluster no.	Cultivars in cluster	Frequency of cultivars in cluster (%)	Mean disease rating ^a	Response class ^b
1	CI 9214, CI 9776 CI 9440, CI 5401, CI 5791, Unitan	30.0	2.5	HR
2	CI 9825	5.0	2.8	R
3	CI 9819, CI 7584, Tifang	15.0	3.2	MR
4	Steptoe, Galt	10.0	4.5	MS
5	CI 5845, CI 9699, Arimont	15.0	6.6	S
6	CI 13727, Dekap, Klages, Clark, Nupana	25.0	7.6	VS

^a Mean disease rating based on average disease ratings of 20–40 plants of each cultivar on a scale of 1–10, where 1 = minute pinpoint or fleck-type lesions, without visible chlorosis and necrosis on the leaf blade; 2 = minute pinpoint or small (1–2 mm long) necrotic lesions, slight chlorosis on the leaf blade, and less than 20% leaf tip necrosis; 3 = small to large (1–2 mm or larger) necrotic lesions, slight chlorosis on the leaf blade, and 21–30% leaf tip necrosis; 4 = small to large necrotic lesions, slight to moderate chlorosis on the leaf blade, and about 31–40% leaf tip necrosis; 5 = small to large necrotic lesions, slight to moderate chlorosis on the leaf blade, and about 41–50% leaf tip necrosis; 6 = coalescing lesions, moderate chlorosis on the leaf blade, and 51–60% leaf necrosis; 7 = coalescing lesions, moderate to severe chlorosis, and 61–70% leaf necrosis; 8 = coalescing lesions, severe chlorosis, and 71–80% leaf necrosis; 9 = coalescing lesions, more necrosis than chlorosis, and 81–90% leaf necrosis; and 10 = coalescing lesions, more necrosis than chlorosis, and less than 10% green area visible on the leaf blade.

^b Response classes: HR = highly resistant, R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible, and VS = very susceptible.

DISCUSSION

Barley is subject to a number of leaf spots including both parasitic and nonparasitic diseases; however, the leaf spot caused by *P. t. f. sp. maculata* might be most easily confused with spot blotch caused by *C. sativus* or an intermediate or resistant reaction to *P. t. f. sp. teres*. Genetic necrosis may also be a confusing factor. Isolation, taxonomic study, and reinoculation are required to verify the disease agent. The Mediterranean isolates of PSB studied in this work appeared slightly different from Montana isolates on the basis of final development of chlorosis and necrosis. Although no isolates from Nepal and Syria were confirmed as PSB, a larger sample may have revealed the disease.

Because preliminary studies revealed 48 of 142 barley cultivars resistant to all isolates of PSB investigated, a number of resistant sources should be available. Because PSB is a relatively newly recognized disease, much more information is required on its distribution, virulence pool, and possible sources of resistance. This investigation extended information in terms of both Montana and the Mediterranean area and pointed out some differences in symptom expression and virulences between PSB isolates in the two areas. The study was not designed for comprehensive detection of all virulence types.

Disease symptoms caused by the PSB organism is not nearly as discrete as with some other host-parasite combinations. Classification of disease was somewhat difficult. Tekauz and Buchanon (13) based disease ratings on the quantity of spores and time required for sporulation. In our study, disease classification emphasis was placed on relative amounts of chlorosis and necrosis. Sporulation was directly associated with these factors, was more readily evaluated, and increased concomitantly with increased chlorosis and necrosis.

It was interesting that both Montana and Mediterranean isolates were either virulent or avirulent on several cultivars in common. The cultivars on which all isolates were avirulent (Unitan, CI 5401, CI 9214, CI 9440, and CI 9776) should be particularly useful as resistant sources in plant breeding programs. Cultivars Arimont, Galt, Steptoe, CI 7584, and CI 9819 showed the most promise for use as

Table 4. Analysis of variance of mean disease ratings of 14 isolates of *Pyrenophora teres* f. sp. *maculata* on 20 barley cultivars

Source	df	Mean square ^a
Cultivars	19	271.9**
Isolates	13	32.6**
Cultivars		
× isolates	247	7.7**
Error	840	0.8

** = Significant at $P = 0.01$.

Table 5. Comparative virulence of Montana and Mediterranean isolates of *Pyrenophora teres* f. sp. *maculata* (Pyrenophora spot blotch [PSB])

Barley cultivar	Mean disease rating					
	All isolates virulent ^a		All isolates avirulent		Isolates with differential virulence	
	MT ^b	MED ^b	MT	MED	MT	MED
Nupana	8.5	7.1
Klages	9.1	5.8
Dekap	8.0	6.2
Clark	7.9	6.2
CI 13727	8.5	5.8
CI 5845	6.9	5.8
CI 9699	7.4	6.3
CI 9825	1.9	4.6
Tifang	1.7	4.0
Unitan	2.3	1.7
CI 5401	2.0	3.2
CI 9214	2.0	1.4
CI 9440	2.4	3.1
CI 9776	2.5	2.4
CI 7584	...	6.0	1.8
CI 9819	...	6.6	1.9
Arimont	6.7	5.4
Galt	5.0	5.2
Steptoe	3.9	4.2
CI 5791	2.9	4.3	...

^aDisease rating greater than 4.3 = virulent.

^bMT = PSB isolates from Montana and MED = PSB isolates from the Mediterranean area.

differential cultivars for determining virulence groups of *P. t. f. sp. maculata*.

Smedegard-Petersen (12) noted that *P. teres* and *P. graminea* might easily cross and produce hybrids with symptoms different from those caused by parental species. It is therefore possible that the various isolates of *P. t. f. sp. maculata* could have evolved through hybridization and mutation.

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Table 6. Frequency of virulence for *Pyrenophora teres* f. sp. *maculata* isolates on 20 barley cultivars

Isolate	Frequency of virulence (%)
Turk 74-Pt6	75
Tun 79-30	65
Tun 79-38	60
Mt Plent82	60
Mt Eddy82-5	55
Mt Lav82	50
Tun 75	50
Mt FF82-12	45
Mt Conrd82	45
Mt Fromb82	45
Mt Lewis82-7	45
Mt FF81E ₁	40
Mt Sun 82-2	35
Mor 82-1	35

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