Twelve *Pyrenophora trichostoma* Races for Virulence to Wheat in the Central Plains of North America

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ABSTRACT

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Forty isolates of *Pyrenophora trichostoma* from the Central Plains of North America were separated into 12 races for leaf spotting virulence on six wheat cultivars. The wheats were separated into six differentials for resistance. BH1146 spring wheat from Brazil was resistant to the largest number of races. These isolates and cultivars are the basis for a system for the determination of genes for virulence and resistance. Larker barley was resistant to all 40 isolates. The fungus also caused "black point" of wheat seed and brown spots on glumes and awns.

Additional key words: Pyrenophora (Helminthosprium) tritici-repentis.

Since 1971, *Pyrenophora trichostoma* (Fr.) Fckl. has been reported as an economically serious pathogen causing "tan spot" or "yellow leaf spot" of wheat leaves in the Central Plains of North America (3–7,18,19,21,22). A synonym of this fungus is *P. tritici-repentis* (Died.) Drechs., and the asexual stage is *Helminthosporium tritici-repentis* Died. (synonyms *Drechslera tritici-repentis* (Died.) Shoem., *H. tritici-vulgaris* Nisikado (5). The fungus is a problem on spring, winter, and durum wheats in many parts of the world (1,2,9,11,12,17,22). It reproduces in lesions on leaves and in straw (3,5,21). It causes black point (2,9) and pink discoloration (17,20) of seed. Its known host range includes thirty-three plant species (15). It infects throughout the growing season, but often has periods of peak activity from wheat heading through harvest (6,13). Wheat residue and older spots on green leaves (5,6), grasses (13,15) and black pointed seed (2) are sources of infective inoculum. Resistance among wheats has been related to the length of the postinoculation period of free water on the foliage (3,6). Procedures for glasshouse screening for resistance (3,6,10) and for laboratory production of conidial inoculum (14–16) have been developed. Variability in virulence has been found in tests with a few isolates (3,12).

The objective of the present investigation was to begin a determination of genes for virulence and resistance by testing for variation of virulence in the fungus with wheats of differing resistance.

MATERIALS AND METHODS

Forty isolates of *P. trichostoma* were obtained from different areas of the Central Plains of North America (3,6,10) (Table 1). Some of the isolation procedures were reported earlier (3,6), and a more recent procedure is described below. Single conidium isolates were obtained from leaf spots by dipping the spotted leaves into 95% alcohol for 15 sec, then into a solution of 1% sodium hypochlorite for 15 sec, and finally rinsing in sterile distilled water (technique attributed to A. Tekauz). One mm pieces of spotted leaf were then placed on V-8 agar (15% V-8, 3.8 g $CaCo_3$ and 25 g agar per liter) in petri plates under 12 hr light and 12 hr dark at 21 ± 3 C for 5 days (14–16). The light was provided by one Norelco F40 WW and one Sylvania F40-BLB fluorescent tube suspended 30 cm above the petri plates. Single conidia were transferred from these plates to petri plates of V-8 agar.

To prepare inoculum for each virulence test, each isolate was grown on standard potato dextrose agar in two petri plates for approximately 10 days at 21 ± 3 C, until the mycelium grew to within a cm of the side of the plates. Then, 1-cm-diameter disks of mycelium and agar, from the young mycelium near the sides of the plates, were placed 10 to a plate on V-8 agar in four petri plates at 21 ± 3 C for 10 days under the above 12 hr light and 12 hr dark (10,14–16); 4 days on V-8 produced better conidiation, but the study was started and therefore continued using 10 days. After 10 days conidia and mycelium were scraped from the surface of the agar in the four plates and blended for 30 sec with 300 ml distilled water in a Waring Blendor. The suspension was placed in a 1-L Erlenmeyer flask and three drops of Tween-20 (polyoxyethylene sorbitan monolaurate) were added to the solution. The inoculum concentration was measured using a hemacytometer and adjusted to 2,000 conidia per milliliter within a final volume of 500 ml.

Based on literature (3,6) and preliminary studies, a group of seven differentially resistant cereal cultivars was selected for inoculation. These cereals were the spring wheat *(Triticum aestivum* L.) cultivars, BH1146 (PI185831), Chris (CI13751), Toropi (PI344200), Duri (PI106301), and ND495, the durum *(Triticum turgidum* L.) cultivar, Wells (CI13333), and the barley (*Hordeum vulgare* L.) cultivar, Larker (CI10648). The plants were grown in an autoclaved soil mixture of two parts Fargo clay loam, one part sand and one part sphagnum in 10.2-cm-diameter clay pots.

The mist chamber for postinoculation wetting of the foliage consisted of four horizontal mist nozzles mounted over a 1 × 4-m glasshouse bench which was enclosed by a transparent, flexible polyethylene film suspended on an aluminum framework 120-cm tall. Plants were misted for 30 sec of each minute with Fargo city water and were constantly wet (3). The glasshouse was maintained at 23 ± 5 C.

Because the mist chamber would accommodate only 140 clay pots and because fungal isolates were collected over a period of several months, each test for virulence contained only five treatments (three fungal isolates, a water/Tween-20 [3 drops Tween-20 in 500 ml water] control and the fungal isolate PyD7 [2,000 conidia plus 3 drops Tween-20 in 500 ml water] as a known virulent standard). Leaves of sixteen plants (four plants per pot) of each of the seven cultivars were dipped in 500 ml of treatment suspension and shaken for 30 sec (3). The plants were at the 4

TABLE 1. Separation of 40 isolates of *Pyrenophora trichostoma* from the Central Plains of North America into 12 races for virulence, wheats into six differentials for resistance, and barley totally resistant

Race	Isolates		Cereal differentials[w]						
	No.	Source[x]	ND495	Chris	Toropi	Wells	Duri	BH1146	Larker
12.	Pti2	h	6.0[y]	5.4	5.2	4.9	4.6	2.5[z]	2.7
	1234CDA	a	6.0	5.3	4.9	3.9	4.1	2.8	2.0
	78-62	f	6.0	5.1	4.5	4.8	3.6	2.4	2.0
	1236CDA	c	6.0	4.0	4.9	3.6	3.5	2.7	1.0
	866	b	5.9	6.0	4.4	4.0	4.5	3.4	2.6
	PyrA-3	i	5.9	6.0	4.7	4.6	3.8	2.1	2.2
	Pyr72	i	5.9	5.5	4.5	4.2	4.6	2.1	2.6
11.	W41	d	6.0	5.2	4.8	4.5	2.6	2.3	2.5
	PyW17	g	5.9	4.9	4.5	4.2	2.4	2.6	2.0
	PTF5	g	5.8	5.5	4.5	4.7	3.0	2.9	2.5
	PTF3	g	5.5	5.0	4.7	4.9	3.3	2.0	2.1
	PyD7	g	5.5	5.0	4.5	3.6	2.7	2.0	2.0
	1235CDA	b	5.3	5.4	4.8	4.9	3.1	2.1	3.0
10.	1231CDA	b	6.0	4.9	3.5	3.4	3.4	3.8	2.4
9.	PTN1	j	5.9	5.5	3.0	4.0	3.9	2.0	2.0
8.	1233CDA	a	6.0	4.5	4.8	2.4	3.0	2.0	1.0
	W37	d	5.9	5.1	3.8	3.3	3.3	2.5	1.0
	W1	d	5.9	4.5	4.3	3.1	2.2	2.0	2.0
	W39	d	5.5	4.9	4.3	2.4	2.5	2.0	1.0
	W42	d	5.5	4.7	4.2	2.5	2.9	2.1	2.3
	PT4	i	5.4	3.6	3.5	3.0	2.8	2.0	1.0
	HP18	e	5.0	4.8	3.7	3.3	3.4	3.3	3.0
7.	PTL1	j	5.2	3.5	2.2	3.6	2.4	3.2	2.0
	W44	d	5.0	4.3	3.3	4.0	3.0	1.9	1.0
	PTD4	g	4.0	3.8	3.0	4.5	2.4	2.0	2.6
6.	PTA3	i	5.2	3.6	2.1	3.1	3.5	2.8	1.0
5.	PTM1	j	5.8	5.0	3.2	2.8	2.7	2.5	2.0
	W38	d	5.5	5.0	2.2	2.0	2.1	2.0	1.0
	W2	d	5.3	5.3	2.8	2.9	2.1	2.0	2.8
	1232CDA	b	4.9	5.5	2.7	2.7	2.3	2.2	3.1
	78-64	f	4.8	4.7	3.0	2.8	2.9	2.0	2.0
	HP28	e	4.6	4.8	3.3	2.8	2.5	2.0	2.4
	HP17	k	4.4	3.5	2.7	2.0	2.3	2.0	2.6
4.	HP8	m	4.5	2.7	3.5	2.1	2.0	2.0	2.2
3.	PyW11	g	4.9	2.6	2.6	2.1	2.0	2.0	2.0
	HP2	m	4.2	3.1	1.8	1.9	1.8	1.7	1.0
	78-65	f	3.9	2.6	2.3	2.2	1.5	1.3	2.4
	HP3A	m	3.5	2.1	1.6	1.6	1.3	1.5	1.0
2.	T1	e	3.2	3.6	2.0	2.2	2.0	1.9	1.0
1.	HP16	l	3.3	2.0	1.5	2.0	1.8	1.5	2.0

LSD ($P = 0.01$) = 0.7 for vertical columns.

[w] Spring wheat—ND495, Chris, Toropi, Duri, and BH1146; durum wheat—Wells; and barley—Larker.

[x] Isolates were obtained: from wheat leaf spots in **a**–Alberta, **b**–Saskatchewan, **c**–Manitoba, and **d**–North Dakota; "from wheat" in **e**–Saskatchewan; as single ascospores from wheat in **f**–Montana, **g**–North Dakota, **h**–South Dakota, and **i**–Nebraska; as single conidia from wheat in **j**–North Dakota; from *Agropyron* sp. in **k**–Saskatchewan; from a *Koeleria* sp. in **l**–Saskatchewan; and from "native grasses" in **m**–Saskatchewan.

[y] Means are based on 12 observations. Rating scale (interpolation between numbers): 1 = no leaf spotting; 2 = less than 2 mm diameter, brown-to-tan spots on <1% of leaf surface; 3 = 2 mm diameter brown-to-tan spots (sometimes with yellow halo) on 3% of the leaf surface; 3.5 = 2–3 mm diameter tan spots, often with yellow halo, on 6% of the leaf surface; 4 = 3–4 mm diameter tan spots, often with yellow halo, on 12% of leaf surface; 5 = greater than 3 mm, coalescing tan spots on 30% of leaf surface; 6 = 50–100% of leaf surface covered with coalesced tan spots.

[z] Ratings 1.0–3.4 indicate avirulence or resistance, 3.5–6.0 virulence or susceptibility (reactions rated as resistant are underlined).

(pseudo-stem beginning to erect) to 6 (first node visible) growth stages of the Feekes and Large scale (8). The resulting 28 pots per treatment were placed in the mist chamber in a completely randomized group and each of the five treatment groups of 28 pots was randomized and separated from the others by a distance of approximately 10 cm.

To distinguish large and potentially important economic differences in cultivar reactions to the various isolates, the following observations and criteria were used. Previous glasshouse studies with isolates W2, W20, PyD7, PyW17, and field-collected conidia had related resistance to tan spot among wheats to duration of the postinoculation foliage wet period. The spring wheat ND495 was severely spotted after only 6 hr postinoculation wetting in mist. Chris required 18 hr, Wells 18–48 hr and some Duri plants 48 hr for severe spotting. All of >3,000 tested wheat selections lost resistance after 54 hr of wetting (3,6). Larker barley remained highly resistant, developing only small necrotic spots, even after 72 hr in mist. Glasshouse resistance related to the field where ND495 was very commonly severely tan spotted, Chris less so, Wells least often and Larker not noticeably (3,4,6,7). Severe tan spotting in the field was related to economic grain losses (1,2,4,6,7). To detect relatively high resistance and yet not obliterate resistance, the present study was conducted with a 30-hr postinoculation wet period. To effect a simple separation into resistant (underlined ratings 1.0–3.4 in Table 1) and susceptible ratings (3.5–6.0 in Table 1), we assumed that the change from insignificant spotting (resistant) to damaging spotting (susceptible) ocurred at around the disease rating 3.5. Significant yield loss did occur in the field above this rating and not below (4,6,7), and the extent of leaf damage became appreciable at about the 3.5 rating (3,4,6,7).

Following the 30-hr misting the plants were fan dried and placed on a glasshouse bench. Severity of leaf spotting was rated 7 days postinoculation by using a scale of 1–6 and interpolating between main classes (Table 1). Tests were considered valid when the isolate PyD7 produced disease severity reactions of at least 5.0, 4.5, and 3.0, respectively, on the cultivars ND495, Chris, and Wells and the water-Tween treated plants were free of spotting (3,6). Three valid tests (replications) were completed with each isolate. The results were statistically analyzed as a split-plot design with isolates as main plots and cultivars as subplots.

RESULTS AND DISCUSSION

Lesions started to become visible on most cultivars 24–48 hr after inoculation. Disease severity was completely expressed and rated 7 days after inoculation.

Highly significant differences in virulence occurred between isolates within cultivars (vertical columns in Table 1). These data supported previous observations of variation in virulence from a few isolates of the organism in North Dakota (3) and India (12). No other conclusions were drawn from the statistical analysis, and susceptible and resistant catagories were set up independent of the analysis.

Larker barley was incorporated into this study to determine if its previously recorded resistance (3) might be overcome by new isolates. Larker was immune or resistant to all 40 isolates (Table 1).

We imposed a nonstatistical race and differential theory upon a statistically significant variation in virulence among isolates. Using the criterion that ratings 3.5 and higher represented virulence, the isolates were separated into 12 races for virulence on the six wheat cultivars. The wheats were separated by the isolates into six distinct differentials. Isolate HP16 from a *Koeleria* sp. was of relatively low virulence on all tested cereals. The breeding line ND495 was susceptible to 10 fungal races (38 isolates). At the other extreme, BH1146 was resistant to 11 races (39 isolates) and Duri to nine races (31 isolates) (Table 1).

While our glasshouse and field observations (3,6,7) suggested the expression of virulence or susceptibility at the 3.5 disease rating and above, and avirulence or resistance below, use of only two categories placed widely separated, statistically different severities of spotting into the same category (Table 1). As we learn more we may want to modify our categories into virulent, intermediate and avirulent. Also, some fungal races were represented by only one isolate (Table 1); the race-differential picture may expand as more material is studied.

However, the above races and differentials can be used to establish gene relationships and improve wheat resistance. Individual fungal isolates from the races can be used to identify genes for resistance in wheat germplasm. Wheat differentials can be used to identify new fungal races. Studies of the genetics of resistance have begun at North Dakota State University and the University of Saskatchewan and are being considered at Montana State University. Tan spot is being generally recognized as a problem in many wheat growing areas, such as the spring and winter wheat areas of North America, parts of Australia and in Nepal. Resistant selections are being exchanged by researchers. From these beginnings a worldwide screening program could develop to obtain and maintain high degrees of resistance to tan spotting in commercial wheats. Although cultural and chemical means are currently used to reduce economic losses from this fungus, increased resistance should be less costly and should assist these controls (4,6,7). Increases in inoculum caused by increasing "minimal tillage agriculture" should make increased resistance even more necessary. The cultivar BH1146 with its resistance to 39 of the 40 fungal isolates might be particularly suited for further resistance studies and incorporation into a breeding program.

Subsidiary findings:

In the course of this study certain other observations were made. The oldest isolate, W1, obtained from a leaf spot in 1968, remained virulent through culture and transfers to the current date. Isolates PyW17, 1235CDA, and PTF3, obtained respectively in 1969, 1976, and 1978, had similar virulence on susceptible wheats. Isolate Pti2, isolated in 1978 from straw collected in 1973, had vigorous growth and the highest virulence among the 40 isolates. These observations indicated that the fungus can maintain its virulence in culture or in the host for long periods. Some isolates, like W1, have remained vigorous and virulent in culture and transfer for years, a few others have declined.

In the subsidiary study at growth stage 10.5.4 (8), 25 heads of the spring wheat breeding line ND495 were inoculated by dip technique with a mycelial and conidial suspension of isolate PTN1 and misted for 30 hr. Seven days after inoculation dark brown spots were evident on the awns and glumes. A few seeds from the inoculated plants showed "black point" symptoms in that the embryos were darkly discolored. Only *P. trichostoma* was reisolated from the spots on the awns and glumes and black point on the seeds when the alcohol-sodium hypochlorite procedure was used. No organisms were isolated from heads and seeds of noninoculated control plants. This suggested that the fungus may be able to infect maturing glumes and awns under severe wet conditions. The fungus was recently found in North Dakota in field-collected durum seed with black point symptoms (F. S. Holbrook, *personal communication*). This is the first report of the fungus in seed in North Dakota.

Cultures of the 40 isolates of this study will be given to the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852.

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