Pathotypes of *Cochliobolus sativus* on Barley in North Dakota

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ABSTRACT

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Spot blotch, caused by *Cochliobolus sativus*, has become an important disease on two-rowed barley cultivars in North Dakota. Information on the variability of virulence in *C. sativus* is essential for the development of spot blotch-resistant barley germ plasm. To establish the number and distribution of *C. sativus* virulence types in North Dakota, 22 isolates from the state were evaluated for their virulence on three differential barley genotypes: ND 5883, Bowman, and ND B112. Eleven additional isolates from Minnesota, South Dakota, Nebraska, Colorado, Japan, and China were included for comparison. Three pathotypes were identified from the thirty-three isolates tested based on the infection responses of the three host genotypes. These pathotypes were designated 0, 1, and 2 according to the coded triplet system of nomenclature. In North Dakota, pathotype 0 (exhibits low virulence on all three differentials) comprised 65%, pathotype 2 (exhibits high virulence on Bowman and low virulence on ND 5883 and ND B112) comprised 25%, and pathotype 1 (exhibits high virulence on ND 5883 and low virulence on Bowman and ND B112) comprised 10% of the total number of barley isolates examined. Within North Dakota, pathotype 1 was found in the east-central and northeastern region only, whereas pathotypes 2 and 0 were distributed throughout the state where samples were taken. Isolates with the pathotype 1 virulence pattern were identified in Colorado, Nebraska, Japan, and China. The remaining isolates collected outside North Dakota were pathotype 0. The information obtained from this study will facilitate the deployment of effective resistance to *C. sativus* in barley.

Additional keywords: *Hordeum vulgare*, virulence diversity

The fungus *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur (anamorph *Bipolaris sorokiniana* (Sacc.) Shoemaker) has an extremely wide host range, including barley, wheat, and rye (10,14). On barley (*Hordeum vulgare* L.), this pathogen causes three diseases: spot blotch, common root rot, and kernel blight or black point (8). Prior to the mid-1960s, spot blotch was especially damaging on barley grown in the upper midwestern region of the United States (8). Yield losses ranging from 16 to 33% have been reported for susceptible barley lines, depending on the environmental conditions (9,17). The importance of spot blotch has decreased over the past 30 years due to the deployment of highly resistant six-rowed malting barley cultivars in the region (11,17). However, the disease still poses a threat to two-rowed barley cultivars, which generally possess lower levels of resistance to spot blotch (3).

Two-rowed barley cultivars are grown for feed in North and South Dakota, and have increased in production area from

13,550 ha in 1985 (1) to 154,350 ha in 1995 (2). Bowman (PI 483237) was the first two-rowed barley cultivar bred specifically for the western Dakotas, and was classified as moderately resistant to spot blotch when it was released in 1984 (4). In 1990, Bowman and lines derived from it became severely infected with spot blotch in a breeding nursery in eastern North Dakota (3). Fetch and Steffenson (3) demonstrated that the resistance in Bowman and some of its progeny was effective against the *C. sativus* isolate ND85F, which has been used routinely in disease evaluations of North Dakota barley germ plasm since 1986, and that the occurrence of another virulence type, expressed by isolate ND90Pr, was responsible for the epidemics observed in two-rowed barley nurseries since 1990. In addition, they (3) demonstrated that isolates ND85F and ND90Pr exhibited differential virulence on several two-rowed barley genotypes. Isolate ND90Pr exhibited high virulence on cv. Bowman and its derivatives and low virulence on barley genotypes ND 5883 and ND 12437. In contrast, isolate ND85F was highly virulent on ND 5883 and ND 12437. Both isolates exhibit low virulence on the six-rowed genotype ND B112 (CI 11531), which is the source of the durable spot blotch resistance present in six-rowed malting cultivars (11,17). Line ND B112, therefore, possesses resistance to both virulence types, a characteristic that has

not been observed in any of the studied two-rowed genotypes.

Disease control by means of host resistance is possible only if the resistance is effective against all virulence types of the pathogen present in the region of crop cultivation. To effectively breed for spot blotch resistance in two-rowed barley germ plasm, it is essential that information be obtained on the variability of virulence in *C. sativus*. Thus, the objective of this study was to establish the number and distribution of *C. sativus* virulence types in North Dakota. In addition, this survey was extended by including some isolates of *C. sativus* from other regions of the United States and from Asia.

MATERIALS AND METHODS

Fungal isolates. Thirty-six isolates of *C. sativus* were evaluated for their virulence phenotype in this study (Table 1). Most were collected during disease surveys of commercial barley fields or breeding nurseries in North Dakota, but at least one isolate was collected from Minnesota, South Dakota, Nebraska, Colorado, Japan, and China. The primary objective of this study was to characterize the virulence of *C. sativus* in its spot blotch phase; thus, most isolates were obtained from the leaves of barley. R002 (provided by R. W. Stack, North Dakota State University, Fargo) was the only isolate derived from roots (wheat) that was evaluated; it was included to compare the virulence of a root isolate with those derived from the foliage. One isolate from rye also was included in the study. Three isolates of known virulence type were used as controls in the study: ND85F, ND90Pr, and ND93-1. The virulence patterns of isolates ND85F and ND90Pr were described previously by Fetch and Steffenson (3). Isolate ND85F induces low infection responses (IRs) on Bowman and ND B112 and high IRs on ND 5883. This pattern contrasts that of isolate ND90Pr, which induces high IRs on Bowman and low IRs on ND 5883 and ND B112. Isolate ND93-1 exhibits low virulence on barley genotypes ND 5883, Bowman, and ND B112 (16). Isolation, storage, and growing conditions for the conidial cultures were as described in Fetch and Steffenson (3), except that minimal medium (15) was used instead of yeast peptone soluble starch agar.

Barley genotypes. The three barley genotypes ND 5883, Bowman, and ND B112 were used in this study because they previously were found to be useful for differentiating *C. sativus* virulence types

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(3). Bowman was derived from the cross Klages/Fergus/Nordic/3/ND 1156/4/Hector (4). The parent Nordic exhibits moderate resistance to *C. sativus*, presumably due to gene(s) originally derived from ND B112. ND 5883 was selected from a cross between Clipper (PI 349366) and the Canadian experimental line 702-10 (3). Line ND B112 was developed at North Dakota State University, Fargo, during the early 1950s and originates from the cross CI 7117-77/Kindred (17).

Evaluation of the seedling infection responses. Each of the 36 *C. sativus* isolates was evaluated for the IRs induced on the barley genotypes ND 5883, Bowman, and ND B112. For this purpose, four to six seeds of each genotype were planted in square pots (12 × 12 cm) filled with a potting mix consisting of peat moss (75%) and perlite (25%). Slow-release (14-14-14, N-P-K, 0.1 g per seedling) and water-soluble (15-0-15, N-P-K, 0.2 g per seedling) fertilizers were applied at planting. All seedlings were grown under growth chamber conditions at 21 to 23°C with a 16-h photoperiod (provided by 115-W cool-white fluorescent bulbs emitting 150 to 350 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$).

Seedlings were inoculated with conidial suspensions (8,000 conidia per ml) of individual *C. sativus* isolates at the two-leaf stage (12 to 14 days old). Conidial cultures for inoculum production were obtained by aseptically transferring silica-gel crystals with adsorbed conidia onto minimal medium in plastic petri plates. After 10 days of incubation at 21°C with a 12-h photoperiod (20-W cool-white fluorescent bulbs emitting 260 to 280 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$), the conidia were scraped from the agar surface with a rubber spatula, suspended in double-distilled water, and filtered through a triple layer of cheesecloth. The concentration of conidia was measured by a hemacytometer and adjusted by adding water. A detergent (polyoxyethylene-20-sorbitan monolaurate) was added to the conidial suspension (100 μl of detergent per liter of suspension) to facilitate the even distribution and adsorption of inoculum onto the leaf surfaces. Inoculum (approximately 0.15 ml per plant) was applied to seedlings with an atomizer (model 15; DeVilbiss Co., Somerset, PA) pressurized (55 kPa) by an air pump. After inoculation, plants were incubated in the dark for 16 h in chambers (21°C with relative humidity near 100%) misted by ultrasonic

humidifiers. Next, the chamber doors were opened to facilitate the slow drying of the plant surfaces. When the plants were dry, they were returned to the growth chamber under the same conditions previously described. The second leaves of seedlings were assessed for their IR 8 to 10 days after inoculation using the 0 to 9 rating scale developed by Fetch and Steffenson (3). With this rating scale, IRs of 0 to 5 are considered indicative of low pathogen virulence and 6 to 9 of high pathogen virulence. All isolates were evaluated for their virulence phenotype in two separate experiments, using a completely randomized design.

Pathotype designation. Pathotypes were designated according to the coded triplet nomenclature system of Limpert and Müller (7). This system uses host differentials ordered in subsets of three. The reaction of each pathogen isolate on a differential host component is first described as a binary value: 1 for virulent and 0 for avirulent (i.e., low virulence). Binary values of each component are further converted to decenary values by the conversion factors 2⁰, 2¹, and 2² for the first, second, and third host differential component in the subset, respectively. Decenary values are summed

Table 1. Isolates of *Cochliobolus sativus* from different counties of North Dakota and other regions tested for their virulence pattern on barley genotypes ND 5883, Bowman, and ND B112

Isolate	Year collected	Geographic origin		Host/part	Host genotype	Row type
		State/country	County/location			
ND85F ^a	1985	ND	Cass	Barley/leaf	Cv. Larker	6
ND90P ^a	1990	ND	Cass	Barley/leaf	Line I86-519-1	2
ND93-1 ^a	1993	ND	Walsh	Barley/kernel	Cv. Robust	6
R002	1976	ND	Walsh	Wheat/root	... ^b	...
ND89-1	1989	ND	Cass	Barley/leaf	...	6
ND89-8	1989	ND	Emmons	Barley/leaf	...	2
ND89-25	1989	ND	Rolette	Barley/leaf	...	6
ND89-33	1989	ND	Mountraill	Barley/leaf	...	6
ND89-37	1989	ND	Dunn	Barley/leaf	...	2
ND89-38	1989	ND	Mercer	Barley/leaf	Cv. Azure	6
ND89-40	1989	ND	Sheridan	Barley/leaf	...	6
ND89R	1989	ND	Cass	Rye/leaf	...	...
SD91-7	1991	SD	Roberts	Barley/leaf	Cv. Robust	6
MN91-11	1991	MN	Traverse	Barley/leaf	Cv. Robust	6
ND92-1	1992	ND	Cass	Barley/leaf	Cv. Robust	6
ND92-2	1992	ND	Cass	Barley/leaf	Cv. Robust	6
ND92-8	1992	ND	Stutsman	Barley/leaf	Cv. Bowman	2
ND92-10	1992	ND	Logan	Barley/leaf	Cv. Bowman	2
ND92-11	1992	ND	Emmons	Barley/leaf	Cv. Bowman	2
NE92	1992	NE	Clay	Barley/leaf	...	6
ND93-11	1993	ND	Cavalier	Barley/kernel	Cv. Robust	6
ND93-12	1993	ND	Ramsey	Barley/kernel	Cv. Robust	6
ND95-17	1995	ND	Towner	Barley/leaf	...	6
ND95-31	1995	ND	Hettinger	Barley/leaf	Cv. Stark	2
KB001	1993	ND	Cass	Barley/kernel	Cv. Chevron	6
KB002	1993	ND	Cass	Barley/kernel	Line M64	6
KB008	1993	ND	Cass	Barley/kernel	Line B2601	6
KB005	1993	ND	Cass	Barley/kernel	Line B2520	6
KB361	1994	CO	Larimer	Barley/kernel	Cv. Steptoe	6
KB362	1994	CO	Larimer	Barley/kernel	Cv. Chevron	6
KB363	1994	CO	Larimer	Barley/kernel	Line B2601	6
KB364	1994	CO	Larimer	Barley/kernel	Cv. Harrington	6
JPN95-1	1995	Japan	Shikoku	Barley/leaf	...	2
JPN95-2	1995	Japan	Shikoku	Barley/leaf	...	2
CHN95-1	1995	China	Hongshan	Barley/leaf	...	2
CHN95-2	1995	China	Hongshan	Barley/leaf	...	2

^a Control isolates previously characterized for virulence patterns on barley (3,16).

^b Information not available or not applicable.

to derive a single number for each subset. The three differential genotypes used in this study were ordered as follows: ND 5883, Bowman, and ND B112. Because only three host differentials were used, a single-digit number was sufficient for the pathotype code of *C. sativus* isolates. The reaction of each isolate on a differential host component was described as 1 for high virulence (IRs 6 to 9) and 0 for low virulence (IRs 0 to 5). For example, isolate ND85F exhibits high virulence on ND 5883 ($1 \times 2^0 = 1$) and low virulence on Bowman ($0 \times 2^1 = 0$) and ND B112 ($0 \times 2^2 = 0$). Thus, the resulting code number for this pathotype is 1.

RESULTS

The IRs induced by the 36 isolates of *C. sativus* on the three barley genotypes were clear and easy to score. Just one or two consecutive (rarely three) IRs were observed on individual plants within each fungal isolate-host genotype combination. The range of IRs observed in each of these combinations was the same in both experiments. The one or two most common IRs observed on the differential hosts to

the *C. sativus* isolates over the two experiments are presented in Table 2. Virulence patterns observed for isolates ND85F and ND90Pr agreed with those described by Fetch and Steffenson (3). The low virulence of isolate ND93-1 on the three barley genotypes (16) also was confirmed. Based on their virulence patterns, isolates ND85F, ND90Pr, and ND93-1 were designated pathotypes 1, 2, and 0, respectively. The other 33 isolates exhibited either low virulence on all three differential host genotypes, or one of two possible low/high virulence patterns on ND 5883 and Bowman, and low virulence on ND B112 as was found for isolates ND85F and ND90Pr. The isolates, therefore, were differentiated into pathotype 0, 1, or 2. No other pathotypes were identified.

All three pathotypes were found in North Dakota: 13 (65%) of the state's barley isolates (from either leaves or kernels) were pathotype 0; 5 (25%) were pathotype 2; and 2 (10%) were pathotype 1. Within North Dakota, pathotype 1 was found only in Cass and Towner counties, whereas pathotypes 0 and 2 were distributed throughout the state where samples were

taken (Fig. 1). The isolates from wheat (root) and rye were both pathotype 0 (Table 2). In addition to North Dakota, isolates with the pathotype 1 virulence pattern were identified in Colorado, Nebraska, Japan, and China. The rest (seven) of the isolates collected outside North Dakota were pathotype 0. Pathotypes 0, 1, and 2 of *C. sativus* were isolated from both barley leaves and kernels in North Dakota. All three pathotypes also were isolated from both row types of barley, although pathotype 0 was isolated more frequently from six-rowed barleys.

DISCUSSION

Three pathotypes of *C. sativus* were identified from 36 isolates based on the IRs elicited on barley genotypes ND 5883, Bowman, and ND B112. These differential hosts were effective for characterizing *C. sativus* virulence because they all gave clear high and low IRs to the pathogen isolates studied (Table 2). This differential set may be useful for determining *C. sativus* pathotypes on a routine basis in regions where spot blotch of barley is a concern. Further refinement of the differential set could be made by developing near-isogenic lines, each differing for a single resistance gene to *C. sativus*. Resistance to pathotypes 2 and 1 in barley genotypes ND 5883 and Morex (a cultivar derived from ND B112), respectively, is governed by a single gene at the seedling stage (11,16). Therefore, it should be easy to develop a set of near-isogenic lines for spot blotch resistance in a common genetic background.

Pathotype 2 was found only among isolates from North Dakota and appears to be more prevalent and widely distributed in the state than pathotype 1 (Fig. 1). It was found in the southeastern (Cass County), south-central (Emmons County), southwestern (Hettinger County), and northeastern (Ramsey County) regions of the state. In the southwestern region of North Dakota, cv. Bowman (susceptible to pathotype 2) is grown in more than 55% of the area planted to barley. Therefore, pathotype 2 should be considered a serious threat to the production of this feed barley cultivar. Part of the breeding effort in the North Dakota two-rowed barley improvement program is aimed at developing low-protein malting barley cultivars for the export market. With success in this endeavor, the production of two-rowed barley cultivars may shift toward the eastern region of the state, where the environmental conditions for spot blotch epidemics are often highly favorable. Thus, it is essential that two-rowed cultivars developed for eastern North Dakota be bred with resistance that is effective against all of the *C. sativus* pathotypes present in the region.

The spot blotch resistance of midwestern six-rowed malting cultivars has remained effective for more than 30 years and is considered durable (11,17). Only one report of

Table 2. The one or two most common infection responses observed on barley genotypes ND 5883, Bowman, and ND B112 infected with 36 isolates of *Cochliobolus sativus*

Isolate	Infection response ^a			Pathotype ^b
	ND5883	Bowman	NDB112	
ND85F	8-9	2-1	2-1	1
ND90Pr	2-3	8-9	2-1	2
ND93-1	2-3	1-2	1-2	0
R002	3	2	2-3	0
ND89-1	2-3	2-3	2-3	0
ND89-8	1	1	1	0
ND89-25	2	2-3	2-3	0
ND89-33	3	3	2-3	0
ND89-37	3	2-3	2-3	0
ND89-38	2	2	2	0
ND89-40	1	2	1	0
ND89R	3	2-3	2-3	0
SD91-7	4-3	3-4	3-4	0
MN91-11	3	3-2	3-2	0
ND92-1	3	2	3-2	0
ND92-2	2	2	2	0
ND92-8	3	9-8	3-2	2
ND92-10	3	8-9	3-2	2
ND92-11	4	7-8	3-4	2
NE92	8	2-3	2-3	1
ND93-11	2	2	2	0
ND93-12	2	8	2-3	2
ND95-17	8-9	2-3	2-3	1
ND95-31	2	8	2-3	2
KB001	2	1-2	1-2	0
KB002	7-8	1	1	1
KB008	2	1-2	1-2	0
KB005	1-2	1-2	1-2	0
KB361	4-5	2-3	2-3	0
KB362	4-5	2	2	0
KB363	2-3	2-3	2-3	0
KB364	5-6	2-3	2-3	1
JPN95-1	7-8	2	2-1	1
JPN95-2	2	2-1	2-1	0
CHN95-1	2-3	2	2-1	0
CHN95-2	8	2-3	2-3	1

^a Observed on 8 to 12 seedlings over two experiments. Infection responses were based on the 0 to 9 scale of Fetch and Steffenson (3).

^b Pathotypes were designated using the coded triplet nomenclature system of Limpert and Müller (7).

Fig. 1. Distribution of *Cochliobolus sativus* pathotypes identified from 23 barley isolates collected in North Dakota. The control isolates of ND85F (pathotype 1) and ND90Pr (pathotype 2) from Cass County and ND93-1 (pathotype 0) from Walsh County are included on the map. Numbers in parentheses denote the number of isolates of the pathotype (if more than one) collected from the particular county.

a *C. sativus* isolate with virulence on ND B112 has been made from North Dakota (5). Although this finding has not been confirmed, it raises serious concerns regarding the future longevity of the ND B112 resistance. Nevertheless, further efforts should be made to incorporate resistance from ND B112 into two-rowed germ plasm. At the same time, *C. sativus* isolates from barley and perhaps other hosts should be monitored in order to detect pathotypes that could potentially threaten the ND B112 source of spot blotch resistance.

All three pathotypes of *C. sativus* were isolated from the leaves of six-rowed cultivars with the ND B112 resistance. This suggests that each pathotype of the fungus is capable of infecting and growing on the foliage of resistant barley cultivars in the field. Keeling and Bantari (6) demonstrated that the net blotch pathogen, *Pyrenophora teres*, could sporulate from small necrotic lesions on resistant barley lines. A similar situation may occur with *C. sativus* on barley.

It is difficult to make any generalizations regarding the tissue specificity of the *C. sativus* pathotypes, because all three virulence types were isolated from barley leaves as well as kernels, and only one root isolate was included in the study. Spot blotch-infected leaves are probably the primary source of inoculum for the kernel

blight phase of the disease; therefore, it is not surprising that similar virulence types were identified from these two tissues. A comprehensive study should be made to determine whether *C. sativus* isolates are specialized to the leaves, roots, or kernels of barley genotypes.

Although only 36 isolates were examined using three differential hosts in this study, *C. sativus* does not appear as variable as two other common foliar pathogens of barley, *Rhynchosporium secalis* (the leaf scald pathogen) and *P. teres*. In Manitoba and Saskatchewan, Tekauz (13) identified 28 pathotypes from 51 isolates of *R. secalis* using 10 differential host lines. *R. secalis* is extremely variable with regard to virulence, even though it has no known sexual stage. The sexual stage of *C. sativus* is rare in nature (14), if it exists at all (8). In contrast, the sexual stage of *P. teres* occurs in some regions of Canada. In a recent virulence survey of *P. teres* f. *teres* in Canada, Tekauz (12) identified 45 pathotypes from 182 isolates using nine differential host lines. Studies on the virulence diversity of fungal pathogens are important. The characterization of pathotypes and their distribution is valuable for programs concerned with breeding for disease resistance. Moreover, this information can provide the foundation for more basic studies on the genetics of host-parasite interactions.

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