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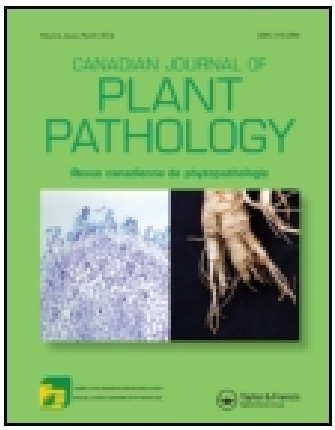

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Identification of *Cochliobolus sativus* isolates expressing differential virulence on two-row barley genotypes from North Dakota

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Severe spot blotch infection was observed in 1990 on several two-row barley breeding lines previously regarded as resistant to *Cochliobolus sativus*. Studies were conducted to compare the virulence pattern of a *C. sativus* isolate (ND90Pr) obtained from this two-row breeding nursery with one (ND85F) used in previous disease screening evaluations. Greenhouse and field experiments were performed in 1991 and 1992 at Fargo, ND, using a split plot design with isolate as the main effect. Isolates ND90Pr and ND85F exhibited distinct differential virulence patterns on barley genotypes ND 5883, ND 12437, ND 12720, ND 12721, and Bowman. Isolate ND90Pr displayed high virulence on ND 12720, ND 12721, and Bowman, and low virulence on ND 5883 and ND 12437. In contrast, isolate ND85F was highly virulent on ND 5883 and ND 12437 and weakly virulent on ND 12720, ND 12721, and Bowman. Both isolates expressed low virulence on genotype ND B112, the primary source of resistance to *C. sativus* in commercial six-row barley germplasm. To incorporate adequate levels of resistance into future two-row barley cultivars, disease evaluations should be made with *C. sativus* isolates that express the full spectrum of virulence found in North Dakota.

Additional index words: Bipolaris sorokiniana, Hordeum vulgare, virulence.

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On a rapporté, en 1990, une grave infection de la tache helminthosporienne chez queques lignées d'orge à deux rangs réputées résistantes au *Cochliobolus sativus*. On a comparé les patrons de virulence d'un isolat (ND90Pr) du *C. sativus* obtenu de cette pépinière de lignées à deux rangs au patron d'un isolat (ND85F) précédemment utilisé pour la sélection de lignées résistantes à cette maladie. Des expériences ont été menées en serre et au champ, en 1991 et 1992, à Fargo, ND; on a utilisé un dispositif en parcelles partagées, ou dispositif en tiroirs, avec les isolats en parcelles principales. Les isolats ND90Pr et ND85F ont présenté des patrons distincts de virulence différentielle chez les génotypes d'orge ND 5883, ND 12437, ND 12720, ND 12721 et Bowman. L'isolat ND90Pr a exprimé une forte virulence chez les génotypes ND 12720, ND 12721 et Bowman et une faible virulence chez ND 5883 et ND 12437. A l'opposé, l'isolat ND85F était très virulent chez ND 5883 et ND 12437 et peu virulent chez les génotypes ND 12720, ND 12721 et Bowman. Les deux isolats étaient peu virulents à l'égard du génotype ND B112, la principale source de résistance au *C. sativus* chez les cultivars commerciaux d'orge à six rangs. Afin d'obtenir des niveaux acceptables de résistance chez les futurs cultivars d'orge à deux rangs, des épreuves de sensibilité au *C. sativus* devraient être réalisées avec des isolats représentatifs de la gamme de virulence retrouvée au Dakota Nord.

Mots-clés additionnels: Bipolaris sorokiniana, Hordeum vulgare, virulence.

The fungus *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dastur (anamorph: *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem.) is the causal organism of spot blotch of barley (*Hordeum vulgare* L.), a disease found wherever the crop is grown (Mathre 1982). In addition to barley, *C. sativus* is also pathogenic on wheat, numerous gramineous species, and even certain dicotyledonous hosts (Kiesling 1985). *Cochliobolus sativus* can attack all parts of the barley plant and causes crop loss by reducing grain yield, decreasing kernel plumpness, and lowering kernel quality by staining the seed coat (Kiesling 1985). In several crop loss studies, yield reductions of 16 to 33% were reported for susceptible barley cultivars due to foliar infection by *C. sativus* (Clark 1979, Nutter et al. 1985, Wilcoxson et al. 1990). Fungicides are often used to increase the yield and quality of barley, but deployment of resistant cultivars remains the best method of disease control against spot blotch (Kiesling 1985).

North Dakota ranks first in barley production among all states in the USA, with an average production (1983–1992) of 3.38 million metric tonnes on 1.13 million hectares (Agricultural Statistics Service 1993). Over 95% of the barley grown in the eastern part (Red River Valley region) of North Dakota is of the six-row type and is used primarily for malting. Two-row barley types, grown mostly in the western half of the state, comprise 17% of the total state production area and are used for feed. The popular six-row barley cultivars grown in North Dakota are all resistant to the foliar phase of the spot blotch disease, presumably due to genes derived from the breeding line ND B112 (Wilcoxson et al. 1990). The resistance derived from ND B112 has remained effective in a number of cultivars since 1964 and is considered durable. Disease evaluations of germplasm from the barley breeding program at North Dakota State University (NDSU) in Fargo indicated that two-row barley genotypes generally possess a lower level of resistance to *C. sativus* than six-row types in greenhouse and field trials (V.D. Pederson, T.G. Fetch, and B.J. Steffenson, unpublished data). Bowman (PI 483237) barley, one of the first two-row cultivars bred for the western Dakotas, was classified as mod-

erately resistant to spot blotch when it was released in 1984 (Franckowiak et al. 1985). However, in 1990, an outbreak of spot blotch was observed on Bowman and its derivatives in test plots in eastern North Dakota. Bowman was derived from a parent with ND B112 in its pedigree, so the occurrence of virulent *C. sativus* isolates on it was of great interest. In this study, we describe the differential virulence pattern of two *C. sativus* isolates, one obtained from a two-row derivative of Bowman barley and the other from the six-row cultivar Larker (CI 10648).

Materials and methods

Fungal isolates. The two isolates of *C. sativus* used in this study were ND90Pr and ND85F. ND90Pr was obtained from leaf samples of a Bowman backcross-derived line (I86-519-1) collected in 1990 at Prosper, ND, and ND85F from leaves of Larker barley collected in 1985 at Fargo. Isolate ND85F was selected for study because it has been used in disease evaluations of barley germplasm at NDSU since 1986, and its virulence pattern was well established. The isolates were obtained in pure culture by the following protocol. Dried leaf samples infected with *C. sativus* were cut into 15- to 20-mm pieces and surface disinfested by immersion in 10% sodium hypochlorite for 60 s. Leaf pieces were rinsed twice with sterile distilled water, blotted dry with a paper towel, and then placed into 9 cm petri dishes with moistened filter paper attached inside the top and bottom halves of the plate. Plates were incubated at 21°C under ambient light. After two days, a single spore was transferred from the respective leaf tissue specimens onto a petri plate containing Yeast Peptone Soluble Starch (YPSS) agar (Tuite 1969). These single spores were the source of the pure culture isolates ND90Pr and ND85F. The plates containing the single spores were placed in an incubator at 21°C with a 12 h photoperiod (260–280 μmol photon·m^{-2}·s^{-1}) for 10 days. The resulting conidia were harvested from the petri plate and adsorbed onto silica gel crystals using a method modified from Tuite (1969). Silica gel crystals were sealed in glass vials and stored at 4°C until needed.

Inoculum preparation. Inoculum was prepared by aseptically transferring 2–3 silica gel crystals with adsorbed conidia of *C. sativus* onto 9 cm petri plates containing YPSS agar and incubating them as previously described. After 10 days of growth, conidia were harvested by adding 20 mL of distilled water and scraping the agar surface with a sterile rubber spatula. The concentrated spore suspension was filtered through three layers of cheesecloth to remove mycelial fragments. Additional water was added to adjust the concentration to either 5000 (greenhouse inoculations) or 8000 (field inoculations) conidia per mL, as measured by using a hemacytometer. A spreader-sticker solution (polyoxyethylene-20-sorbitan monolaurate) was added to the conidial suspension (100 μL per L) to facilitate the even dispersal of inoculum onto the leaf surfaces.

Barley germplasm. The virulence phenotypes of the two *C. sativus* isolates were evaluated on seven barley genotypes: Bowman, ND B112, Stark, ND 12720, ND 12721, ND 5883, and ND 12437. With the exception of ND B112, all genotypes were two-row types. Bowman was derived from the cross Klages/Fergus/Nordic/3/ND1156/4/Hector (Franckowiak et al. 1985). The parent Nordic exhibits moderate resistance to *C. sativus*, presumably due to gene(s) originally derived from ND B112. Stark, ND 12720, and ND 12721 were derived from crosses to Bowman. The genotype ND 5883 was selected from a cross between Clipper (PI 349366) and the Canadian experimental line 702-10 (J.D. Franckowiak, personal communication). ND 12437 was produced from a cross between ND 7691 and ND 9939 (a selection from a Bowman sib × ND 7544 cross). These genotypes were selected because they exhibited distinct differential responses to the two pathogen isolates in a previous greenhouse screening test.

Greenhouse studies. Barley lines (4–5 seeds) were planted in plastic cones (20.7 cm depth and 3.8 cm diameter) containing a 75% peat moss:25% perlite potting mix. A slow-release fertilizer (14-14-14, N-P-K) was added to each cone at a rate of 1.0 g per cone. The planted potting mix was then watered with a water-soluble fertilizer (15-0-15, N-P-K, 536 ppm N rate). Plants were grown in a greenhouse at 23 ± 2°C and 14 h photoperiod, with supplemental lighting provided by 1000 W metal halide lights (530–710 μmol photon·m^{-2}·s^{-1}). The experimental design was a split-plot (isolate = main plot and genotype = subplot) using a randomized complete block arrangement with 10 replicates. Greenhouse experiments were conducted in 1991 and repeated in 1992.

Seedlings were inoculated with the conidial suspension at the two leaf stage (12–14 days old). Inoculum was applied at a rate of approximately 0.2 mL per plant using an atomizer pressurized by an air pump at 55 kPa. After inoculation, plants were placed in chambers (21°C and 95–100% RH for 16 h) misted with ultrasonic humidifiers (Steffenson & Fetch 1990) in the dark. Next, the chamber doors were opened to allow the plants to slowly dry off. Plants were then returned to the greenhouse under the same conditions described above.

The seedling infection phenotype (IP) was assessed on the second leaves of plants 12 days after inoculation. Although a disease rating scale for evaluating *C. sativus* infection phenotypes on barley seedling leaves has been published (Cook & Timian 1962), it

does not cover the full range of types that were observed in our studies. Thus, a 0–9 scale was developed (Table 1). Infection phenotypes 0–4 were considered indicative of low pathogen virulence (low IP), 5–6 of intermediate virulence (intermediate IP), and 7–9 of high virulence (high IP).

Field studies. Isolates ND90Pr and ND85F were also evaluated for their virulence pattern on the same seven barley genotypes in field trials conducted in 1991 and 1992. Genotypes were hand planted in hill plots (six replicates) using the experimental design previously described. Within a replicate, two paired main plots were planted, one for each isolate of *C. sativus*. Hills of the seven genotypes were planted in two rows as subplots. The spacing between hills and rows was 0.3 m. To reduce interplot interference, border strips of Karl (PI 559373) triticale (resistant to *C. sativus*) were planted around the main plots of the barley test entries in each replicate using a four-row cone-planter with 0.3 m row spacing. The distance between main plots within a replicate was 2 m and between replicates 100 m. Plots were established in an area that had not been previously planted to small grains for three years in order to reduce contamination of plots by infective propagules of *C. sativus* from the soil. When the flag leaf was fully extended, plants were inoculated with conidia of isolate ND90Pr or ND85F at a rate of 50 mL per hill. These inoculations were made on a calm, clear evening when the likelihood of dew formation was great. No spot blotch infections were observed on plants prior to inoculation.

Five randomly selected tillers from each hill were assessed for disease severity (percentage of leaf tissue infected, including both necrotic and chlorotic areas) and infection response (size and type of lesion) at weekly intervals for three weeks, beginning 12 days after inoculation. This time period corresponded to the late milk through late dough stages of plant development. Disease severity was assessed using standard area diagrams (James 1971). The scale used for classifying adult plant infection responses was based on a generalized scale developed by MacNeal et al. (1971): R = resistant (necrotic lesions <2 mm long with no chlorosis); MR = moderately resistant (necrotic lesions 2–4 mm long with no chlorosis or marginal chlorosis); MS = moderately susceptible (necrotic lesions 4–6 mm long surrounded by a definite chlorotic ring); and S = susceptible (necrotic lesions >6 mm long with extensive chlorosis). Data on disease severity were analyzed using the SAS General Linear Models (GLM) procedure (SAS Institute, Inc. 1989).

Randomly selected leaf samples (5 leaves per hill, 5 hills per main plot) were collected from all replications in 1991 and 1992 to verify that the isolate originally inoculated was responsible for causing the disease symptoms observed in the respective main plots. The virulence of these samples was evaluated on seedlings of the same seven barley genotypes as previously described. Although some exchange of inoculum between main plots occurred, the vast majority of lesions on host genotypes were due to the original inoculated isolate. Only 16% of isolates sampled from lesions on all host genotypes did not exhibit the virulence pattern of the original inoculated isolate.

Results

Greenhouse experiment. Isolates ND90Pr and ND85F exhibited distinct differential virulence responses on several barley genotypes (Table 2). Isolate ND90Pr exhibited high IPs (7-8) on Bowman, ND 12720, and ND 12721, and low IPs (4-3) on ND 5883 and ND 12437. In contrast, isolate ND85F exhibited low IPs (3-2, 2-3) on Bowman, ND 12720, and ND 12721, and high IPs (8-7, 7-8) on ND 5883 and ND 12437. Isolate ND90Pr and ND85F exhibited intermediate (IP 6-5) and intermediate to high virulence (IP 7-6) on the cultivar Stark, respectively.

Table 1. Description of infection phenotypes (IPs) observed on barley seedlings infected with *Cochliobolus sativus* in greenhouse experiments (1991–1992)

Infection phenotype	Description of lesion size and type	
	Necrotic area† (mm)	Chlorosis
0	None	Immune, no lesions observed
1	<1	No chlorosis observed
2	1–2	No chlorosis observed
3	1–2	Diffuse chlorotic ring (<0.25mm)
4	2–3	Chlorotic ring (<0.50 mm), or halo
5	3–4	Chlorotic ring (0.50–0.75 mm)
6	4–6	Chlorotic ring (0.50–0.75 mm)
7	6–7	Chlorotic ring expanding in length (0.75–1 mm) and width (0.75–1 mm)
8	7–8	Chlorotic ring expanding in length (1-1.5 mm) and width (1–1.25 mm)
9	>8	Chlorotic ring expanding in length (1.50-2 mm) and width (1.25–1.50 mm)

†For infection phenotypes (IPs) 1 and 2, necrosis is the diameter (mm) of the necrotic spot; for IPs 3–9, necrosis is the length (mm) of the necrotic lesion.

Table 2. Infection phenotypes (IPs) of barley genotypes infected with isolates ND90Pr and ND85F of *Cochliobolus sativus* in the greenhouse at 23 ± 2°C

	Isolate			
	ND90Pr		ND85F	
Barley genotype	Mode†	Low/High‡	Mode	Low/High
ND B112	3-2	2/4	3-2	2/4
ND 5883	4-3	2/5	8-7	7/9
ND 12437	4-3	3/5	7-8	6/9
Bowman	7-8	6/9	3-2	2/4
ND 12720	7-8	6/9	3-2	2/4
ND 12721	7-8	5/8	2-3	2/4
Stark	6-5	4/7	7-6	5/8

†Mode represents the two most frequent IPs, in decreasing order of abundance, found over all replicates (1991 and 1992, pooled data).
‡Low/High represents the lowest and highest IPs observed over all replicates (1991 and 1992, pooled data).

Both isolates displayed low virulence (IP 3-2) on the resistant check ND B112.

Field experiment. Disease severity (DS) and adult infection responses (IRs) of barley lines infected with isolates ND90Pr and ND85F are presented in Table 3. Disease reactions were consistent on host genotypes over the two-year study, although plant growth and disease progress were slower in 1992 due to a cooler than average growing season. Both *C. sativus* isolates exhibited low virulence (DS: 5.3–7.5% and IRs: R-MR) on line ND B112. Isolate ND90Pr exhibited relatively low virulence on lines ND 5883 and ND 12437 (DS: 19.5–28.3% and IRs: MR-MS) and high virulence (DS: 78.8–93.3% and IRs: S-MS) on Bowman, ND 12720, and ND 12721. In contrast, isolate ND85F expressed high virulence (DS: 49.2–70.8% and IRs: MS-S) on lines ND 5883 and ND 12437, and low virulence (DS: 11.5–22.5% and IRs: mostly MR, with some MS) on Bowman, ND 12720, and ND 12721. Isolate ND90Pr exhibited intermediate virulence (DS:29.2% and IRs: mostly MS with some S), and isolate ND85F intermediate to high virulence (DS: 39.2% and IRs: MS-S) on Stark in 1992. Analysis of disease severity data revealed significant isolate × host genotype interactions (1991: $P = 0.0001$, 1992: $P = 0.0006$, Pooled: $P = 0.0453$) as well as significant differences between isolates and between cultivars (1991: $P = 0.0001$, 1992: $P = 0.0001$, Pooled: $P = 0.0001$, same values for both variables). These results confirmed the differential virulence patterns of isolates ND90Pr and ND85F on barley in field studies.

Discussion

The objective of this study was to compare the virulence pattern of a *C. sativus* isolate (ND90Pr) responsible for causing a high level of disease on Bowman barley and its derivatives to one (ND85F) routinely used in disease resistance evaluations at NDSU. The data presented clearly indicate that isolate ND90Pr is markedly different from ND85F in virulence on selected two-row barley genotypes. Although some exchange of inoculum occurred between main plots inoculated with different isolates in the field, the results closely agree with experiments conducted on seedlings in the greenhouse. In the field, differential reactions were noted not only for infection response, but also for disease severity as indicated by the significant isolate × genotype interaction term.

Bowman, a cultivar with ND B112 in its pedigree, was bred for the western regions of North Dakota where the average annual rainfall is about 40 cm. The spot blotch pathogen is favored by warm, humid environmental conditions, and in wet years it can be a

Table 3. Disease severity (DS) and infection response (IR) of barley genotypes to isolates ND90Pr and ND85F in 1991 and 1992 at Fargo, ND

	1991				1992			
	ND90Pr		ND85F		ND90Pr		ND85F	
Genotype	DS†	IR‡	DS	IR	DS	IR	DS	IR
ND B112	5.8	R–MR	7.5	R–MR	5.3	R–MR	5.8	R–MR
ND 5883	28.3	MR(MS)	70.8	MS–S	20.8	MR(MS)	70.0	S–MS
ND 12437	27.5	MR–MS	57.5	MS–S	19.5	MR(MS)	49.2	S–MS
Bowman	78.8	MS–S	16.7	MR	83.2	S–MS	11.5	MR(MS)
ND 12720	87.2	MS–S	22.5	MR	90.0	S–MS	14.2	MR(MS)
ND 12721	93.0	MS–S	15.0	MR	93.3	S–MS	13.7	MR(MS)
Stark§					29.2	MS(S)	39.2	MS–S
LSD (0.05)	13.8		16.2		13.3		11.3	

†DS is the average disease severity (percentage of leaf tissue affected) of six replicates at the late dough stage of development.
‡IR(s) is/are the most common infection response(s) observed where R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible. IRs denoted in parentheses were found in low frequency (<5%) in test plots.
§Stark was not planted in field trials in 1991.

problem in the western growing areas. When Bowman was released in 1984, it was regarded as being moderately resistant to *C. sativus* (Franckowiak et al. 1985). Since 1990, Bowman and lines derived from it have been severely infected with spot blotch in breeding nurseries located in the Red River Valley, a region with generally higher humidity and precipitation. These two-row genotypes probably lack the full complement of spot blotch resistance gene(s) present in ND B112, since six-row genotypes that also possess the ND B112 resistance have not displayed any susceptibility to *C. sativus*.

Since virulence surveys of *C. sativus* had not been conducted previously, it is difficult to state with certainty whether isolates with the ND90Pr virulence pattern were already present in eastern North Dakota (albeit at low frequency) prior to 1990 or arose more recently via mutation. New virulence types are not thought to arise via sexual reproduction, because the perfect stage has not yet been reported in nature (Tinline 1988). Christensen (1925) described virulence in *C. sativus* as "dynamic" because isolates readily sectored out when grown on artificial media, and the virulence pattern of the mutant sectors was different from the original isolate. Isolates of *C. sativus* that vary in virulence on barley (Clark & Dickson 1958, Wood 1962) and on wheat (Misra 1979) have previously been reported. However, prior to 1985 there was no conclusive evidence for differential virulence of *C. sativus* on barley, (Dostaler et al. 1981, Gayed 1962, Tinline 1954), though it was thought to exist (Clark 1956). Recently, Levitin et al. (1985) found host-specific virulence in isolates of *C. sativus* obtained from diverse regions in Russia. In this study, we confirmed the occurrence of host-specific isolates of *C. sativus* in North Dakota.

The discovery of isolates possessing host-specific virulence is important because resistant cultivars are used as the primary control method for this pathogen. Information regarding the genetics of resistance in barley, and virulence in *C. sativus*, would be useful in the development of effective gene deployment schemes. Currently, the number and distribution of *C. sativus* virulence types in North Dakota is unknown, but evaluation of stored cultures (isolates collected prior to and after 1990) are underway. Two-row germplasm from North Dakota remains vulnerable to damage by *C. sativus*. This is in contrast to the situation with six-row germplasm where adequate levels of spot blotch resistance have been maintained in cultivars since 1964. To develop two-row germplasm with adequate levels of spot blotch resistance, disease evaluations should be made with isolates ND90Pr, ND85F, and perhaps others if additional virulence types are identified.

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