First Report of Scald of Triticale Caused by *Rhynchosporium secalis* in North America

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ABSTRACT

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Scald, caused by *Rhynchosporium secalis,* was found for the first time on leaves of triticale in the Willamette Valley in Oregon in April 1995. Koch's postulates were completed and four separate inoculation studies were done with 12- to 17-day-old greenhouse-grown seedlings of triticale lines. Seedlings were examined beginning 3 days after inoculation and at 2- to 3-day intervals until a final rating was made 11 to 20 days after inoculation. Nine to 15 days after inoculation, leaves of susceptible plants of triticale lines 149 TR 3-232, 83 TP 1-121, and 191 TR 2-12 lost turgor, wilted, and collapsed, changing from green to gray to chlorotic. No leaf lesions developed in triticale lines 5735 TW 3-324 and 431 TU 1-22. Seedlings of rye, winter and spring wheat, and barley were also inoculated with conidia of *R. secalis*. Severe scald developed in rye cvs. Prima and Jingshou and rye semidwarf line SD-152. Some cultivars of inoculated barley developed small lesions along margins of leaves, but were considered resistant to scald. No disease symptoms developed in inoculated wheat. Symptomatic leaf sheaths and blades from triticale line 149 TR 3-232 were dried and deposited in the Herbarium, Department of Botany and Plant Pathology, Oregon State University, Corvallis. A culture of *R. secalis* from triticale in Oregon was deposited in the American Type Culture Collection, Rockville, MD, as ATCC 96698.

Triticale (× *Triticosecale* Wittmack) is a small-grain cereal resulting from an intergeneric hybridization of wheat (*Triticum)* and rye (*Secale*) (7). Its grain is commonly used for animal feed, and green plants are used as forage or silage. An estimated 2.5 million ha are grown in Western and Eastern Europe, South America, Africa, China, Australia, and India; about 250,000 ha of triticale are grown in the U.S., 15,000 ha in Canada, and 5,000 ha in Mexico (Wolfgang Pfeiffer, personal communication).

In mid-April 1995, a serious foliar disease was observed in leaf blades of triticale line 149 TR 3-232 in a yield trial near Corvallis, OR (Fig. 1). Leaf spot diseases are common in small grains grown in the Willamette Valley, but this disease was not recognized from its macroscopic symptoms. Leaves of 22 other lines of triticale grown in the same yield trial were asymptomatic. The purpose of this study was to determine the cause of this leaf spot disease, document its occurrence, and perform a limited host range study.

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MATERIALS AND METHODS

Sample collection. Leaf blades showing leaf spot symptoms were collected from triticale growing in the yield trial and cut into small pieces (1 to 2 cm) that included lesions with healthy tissue margins. Leaf pieces were surface sterilized in 70% ethanol for 2 min, rinsed with deionized water, surface sterilized again in 1% NaOCl for 1 min, rinsed again with sterile deionized water, and transferred aseptically to petri dishes containing 2% agar acidified with lactic acid (AWA). Dishes were incubated in a temperature-controlled cabinet to provide 12 h of dark at 10°C and 12 h in light (50 $\mu E \cdot s^{-1} \cdot m^{-2}$) at 15°C on a 24-h cycle. Leaf pieces were examined at ×100 to ×160 every 2 to 3 days.

Twelve days after incubation, a slow-growing, yeastlike fungus was observed on leaf spot lesions and into AWA. Portions of the matrix were transferred into drops of distilled water and examined at ×160 to ×400. Other portions of the matrix were transferred aseptically to petri dishes and test tubes containing potato dextrose agar (PDA) and incubated 15 days in a controlled environment chamber described previously. All cultures that developed on PDA were identical, and five pure cultures were obtained and stored at 3 to 5°C. During the study, one stock culture was used to produce inoculum on PDA for inoculating test plants.

Plant growth procedures. Two seeds of each breeding line or cultivar of triticale, cereal rye (*Secale cereale* L.), winter wheat (cv. Gene, PI 560129) and spring wheat (cv. Penewawa, PI 495916) (*Triticum aestivum* L.), or barley (*Hordeum vulgare* L.) were sown into 12 to 14 cone-shaped plastic containers (3.8 × 21 cm) containing fine-grade vermiculite supplemented with 3 to 6 g of osmocote fertilizer (20 20 20) per cone. Containers in plastic racks were incubated in a greenhouse at 20 ± 5°C, with supplemental light programmed to provide a minimum 12-h photoperiod. Containers were watered twice daily to maintain plant growth.

Inoculation and incubation. Pure cultures of the fungus were grown on PDA or acidified PDA. Deionized water was added to the culture dish, the surface of the colony was rubbed to dislodge conidia, and the suspended conidia were counted in a hemacytometer. The suspension was sprayed onto foliage of test plants and inoculated seedlings were placed in a dew chamber for 72 to 96 h, with alternating cycles of 24 h at 14°C in the dark and 24 h in continuous light (intensity front to back ranged from 110 to 30 $\mu E \cdot s^{-1} \cdot m^{-2}$) at 19°C. Halfway through the light cycle, racks were rotated 180 degrees to provide light to opposite sides of each plant. Plants were returned to the greenhouse and examined for disease symptoms 3 days after inoculation and at 2- to 3-day intervals until a final disease assessment was made 11 to 20 days after inoculation. Culture age, concentration of conidia, seedling age, and cultivars and lines inoculated are described with each test.

Host range tests. In test 1, a conidial suspension in water (concentration undetermined) was sprayed onto leaves of 12-

day-old seedlings of triticale line 149 TR 3-232, the entry showing severe leaf spot symptoms in the field, barley (cv. Steptoe and line HM 123), winter wheat (cv. Gene), spring wheat (cv. Penewawa), and rye (cv. Jingshou and line SD 152). With one plant of each entry in a single container, the test was arranged as a randomized complete block (RCB) with 7 entries and 14 replications. Two noninoculated plants of each entry (i.e., 14 plants) served as controls. The study contained 112 plants (7 entries × 16 containers).

In test 2, a conidial suspension (3×10^5 conidia/ml) from a 32-day-old culture of the fungus growing on PDA was sprayed onto foliage of 16-day-old seedlings from each of four triticale lines (149 TR 3-232, 191 TR 2-12, 83 TP 1-121, and 5735 TW 3-324), and triticale cv. Colina, barley cv. Harrington, and rye cv. Prima. The test was arranged in an RCB with 7 entries and 12 replications. Two noninoculated plants of each entry served as controls.

In test 3, a conidial suspension (6.5×10^5 conidia/ml) from a 37-day-old culture of the fungus growing on PDA was used to inoculate foliage of 17-day-old seedlings of four lines of triticale (149 TR 3-232, 191 TR 2-12, 83 TP 1-121, and 5735 TW 3-324), triticale cv. Colina, rye cv. Prima, and barley line KB941055. This study was arranged as an RCB with 7 inoculated entries in 12 replications and 7 noninoculated entries in 2 replications

In test 4, 14-day-old seedlings of four cultivars of barley, (Steptoe, Harrington, KB941055, and Morex) and two lines of triticale (149 TR 3-232 and 431 TU 1-22) were inoculated with a conidial suspension (4×10^6 conidia/ml) from a 14-day-old culture of the fungus growing on PDA. Container were sown with 2 seeds of each entry, but not all seeds germinated. The study was arranged in an RCB with 6 inoculated entries in 10 replications and 6 noninoculated entries in 4 replications.

Seeds of the six triticale lines were originally supplied by G. H. Ittu, Research Institute for Cereals and Industrial Crops, 8264 Fundulea, Jud. Calarasi, Romania, for use as sources of earliness in the triticale breeding program at Corvallis, OR. Jingshou rye originated in western China, Prima rye was developed in Canada, and SD-152 is a semidwarf line. HM 123 and KB941055 are lines of barley supplied by Mathias Kolding, Oregon State University; cvs. Septoe, Harrington, and Morex are common cultivars of barley in the U.S. Gene and Penewawa are hexaploid winter and spring wheats, respectively.

RESULTS

Pathogen identification. White, opaque, yeastlike colonies developed in leaf lesions from the field. Conidia that developed in subcuticular stroma were hyaline with a single septum and a slight beak on one end (Fig. 2). Conidia from five different locations on a leaf were suspended in distilled water and 25 were measured at ×160 to ×400 with an ocular micrometer. Conidia means were 16.3 µm (range 12.5 to 23.8 µm) × 5.4 µm (range 5 to 7.5 µm) and were similar to conidia from rye (8) and typical of *Rhynchosporium secalis* (Oudem) J. J. Davis (9). The fungus grew slowly on PDA with colonies reaching a diameter of 2 to 3 mm in diameter after 4 weeks of incubation at 10°C (12 h light) and 15°C (12 h dark) on a 24-h cycle.

Host range tests. In the first test, inoculated leaf blades of triticale (Fig. 3) and rye lost turgor, turned from green to gray, wilted, and collapsed 11 days after inoculation. Leaf tissue with lesions was collected, surface sterilized as previously described, and cultured on AWA. *R. secalis* was reisolated from inoculated triticale and rye, thus completing Koch's postulates. Necrotic lesions developed on leaf margins of inoculated barley cv. HM 123, but inoculated barley cv. Steptoe was free of leaf lesions. No lesions developed on inoculated leaves of either winter or spring wheat, or on noninoculated controls.

In the second test, large lesions developed in inoculated leaves of triticale lines 149 TR 3-232 and 83 TP 1-121 and rye cv.

Fig. 1. Spike of triticale line 149 TR 3-232 showing variations in leaf lesions on three upper-most leaves.

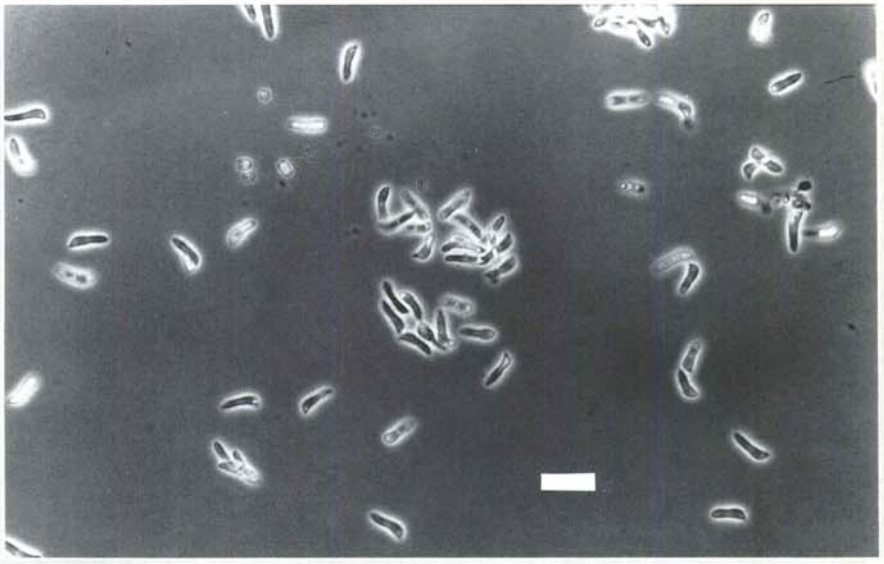

Fig. 2. Conidia of *Rhynchosporium secalis* in distilled water collected from leaf lesions in triticale (scale bar = 20 µm).

Prima. No lesions developed in inoculated leaves of triticale line 5735 TW 3-324, but lesions developed in leaves of one plant in line 191 TR 2-12 and cv. Colina. Small lesions developed along margins of inoculated leaves of barley cv. Harrington. Two noninoculated plants of each entry were used as controls and remained symptomless.

In the third test, scald was severe (all inoculated plants were infected) in triticale lines 149 TR 3-232 and 83 TP 1-121 and rye cv. Prima. Five of 14 inoculated plants of triticale line 191 TR 2-12 were also infected by *R. secalis*. No leaf symptoms developed in inoculated triticale line 5735 TW 3-324, triticale cv. Colina, barley line KB941055, or noninoculated controls.

A fourth test was done primarily to evaluate scald reaction in barley cvs. Steptoe, Harrington, and Morex, and breeding line KB941055. The barley breeder developing KB941055 reports this line is highly susceptible to scald in field tests (Mathias Kolding, personal communication). Two lines of triticale, 149 TR 3-232 as a susceptible check, and 431 TU 1-22, a semidwarfdwarf line, were included in the test. Fifteen days after inoculation, typical scald symptoms developed in triticale line 149 TR 3-232. No symptoms of scald developed in inoculated leaves of the other cultivars and lines, nor in the noninoculated controls.

DISCUSSION

A literature search of the Commonwealth Agricultural Bureaux (1985 to present), BIOSIS (1969 to present), and AGRICOLA (1979 to 1984) was done on 15 May 1995. No citations or references were found for scald or *R. secalis* in triticale. Two host indices (4,5) consulted did not list triticale as a host for *R. secalis*. Scientists at the Centro Internacional de Mejoramiento de Maiz Y Trigo (CIMMYT), Mexico, D.F. Mexico, were contacted for information about the occurrence of this disease. *Rhynchosporium secalis* in triticale has been observed in Europe (1,11, 12), Kiev, Ukraine in 1991 (E. E. Saari, personal communication), Chile (Wolfgang Pfeiffer and Lucy Gilchrist, personal communication), and Morocco (Mohamed Mergoum, personal communication). No other occurrences of this disease were noted by individual scientists or in the formal scientific literature.

Based on disease assessments (number of infected plants) with one isolate of *R. secalis,* and under the conditions of these tests, two lines of triticale were susceptible, two lines were resistant, and one line was heterogeneous (which suggests a mixture of genotypes in the host) in their response to scald. Of the other small grains inoculated with *R. secalis* from triticale, cereal rye was susceptible; barley and winter and spring wheat were resistant. Host specificity has been shown in cross-inoculation studies of gramineous hosts with isolates of *Rhynchosporium secalis* from Argentina, Australia, Britain, and the U.S. (3,8). In this study, an absence of scald symptoms in barley inoculated with conidia from this isolate of *R. secalis* is likely the result of host specialization.

Tetraploid and hexaploid wheats, sources of the A and B genomes in triticale, are nonhosts for *R. secalis*. In contrast, rye is very susceptible to *R. secalis*. We did not expect these triticale lines to be susceptible to *R. secalis,* but based on results obtained in this study, susceptibility of triticale to *R. secalis* most likely was due to the absence of scald resistance on the A or B genome supplied by the wheat parent. Because most triticale lines are resistant to *R. secalis* and most ryes are susceptible, it is highly unlikely that rye is a major source of resistance to *R. secalis* in triticale. In most triticale lines, resistance contributed by wheat is apparently adequate to protect them from scald.

Susceptibility of the triticale lines listed in Table 1 may be due to one or more of the following reasons: (i) loss of the wheat resistance genes through chromosome loss (aneuploidy, translocation, or substitution); (ii) genes from wheat may have mutated to an inactive state; (iii) genes from wheat may have been inactivated by some interaction(s) between wheat and rye genomes; or (iv) genes that control resistance in A/B genomes were overcome by this previously unknown pathogenic strain of *R. secalis*.

Histopathological studies on *R. secalis* infection of barley (2) and *R. orthosporium*

Fig. 3. Lesions of *Rhynchosporium secalis* in inoculated leaves of triticale.

Table 1. Response of 12- to 17-day-old seedlings of selected cereal grains inoculated with *Rhynchosporium secalis* isolated from triticale breeding line 149 TR 3-232

Species	Cultivar or breeding line	No. of seedlings with severe leaf symptoms and no. inoculated				Disease assessment
		Test 1	Test 2	Test 3	Test 4	
Triticale	149 TR 3-232	14 of 14	11 of 12	14 of 14	13 of 16	Susceptible
	83 TP 1-121	. . .	12 of 12	14 of 14	. . .	Susceptible
	5735 TW 3-324	. . .	0 of 12	0 of 14	. . .	Resistant
	431 TU 1-22	. . .	. . .	. . .	0 of 17	Resistant
	191 TR 2-12	. . .	1 of 12	5 of 14	. . .	Heterogeneous
	Colina	. . .	1 of 12	0 of 14	. . .	Resistant
Rye	Jingshou	14 of 14	. . .	. . .	. . .	Susceptible
	Prima	. . .	11 of 11	14 of 14	. . .	Susceptible
	SD-152	14 of 14	. . .	. . .	. . .	Susceptible
Barley	Steptoe	0 of 14	. . .	. . .	0 of 19	Resistant
	HM 123	Margin[a]	. . .	. . .	. . .	Resistant
	Harrington	. . .	Margin[a]		0 of 17	Resistant
	KB941055	. . .	. . .	0 of 14	0 of 19	Resistant
	Morex	. . .	. . .	. . .	0 of 18	Resistant
Wheat	Gene	0 of 14	. . .	. . .	. . .	Resistant
	Penawan	0 of 14	. . .	. . .	. . .	Resistant

[a] Small lesions developed in inoculated leaves along leaf margins.

infection of orchardgrass (*Dactylis glomerata* L.) (10) established that the fungus penetrates the host between contiguous epidermal cells or between guard or subsidiary cells. Two or 3 days after penetration, subcuticular mycelium develops intercellularly between the cuticle and epidermal cells. Five to 6 days after inoculation, mycelium invades mesophyll cells, and by 7 to 10 days, symptoms of scald appear on the leaf blade. Finally, the mesophyll cells are colonized and disintegrated by intracellular mycelium. If infection of triticale by *R. secalis* is similar to *Rhynchosporium* infection of barley and orchardgrass, it suggests resistance to scald in triticale, which is most likely provided by genes in A or B genomes of wheat, is expressed in epidermal cells.

Masses of conidia from the original culture of *R. secalis* were used to prepare inoculum for each test. It is well established in barley that considerable pathogenic variability exists among single conidial cultures of *R. secalis* and this variability can be a serious problem in evaluating resistance to scald (6). Single-conidia cultures were intentionally not used, to avoid limiting pathogenic variation in this study of host responses.

A culture of *R. secalis* from this study was deposited in the American Type Culture Collection (No. ATTC 96698) and dried plant specimens were placed in the Herbarium, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331.

LITERATURE CITED

1. Abreu, C. A. 1979. Fungal diseases on triticale wheat and rye in Vila Real (1979). Melhormento: Estudos da Estacao de Melhoramento de Plantas (Portugal) 27:241-246 (in Portuguese with English summary) in: Wheat, Barley, Triticale Bibliography 1:33, Pub. No. 00591, 1985, CIMMYT, El Batan, Mexico.
2. Ayesu-Offeu, E. N., and Clare, B. G. 1970. Processes in infection of barley leaves of *Rhynchosporium secalis*. Aust. J. Biol. Sci. 23:229-307.
3. Caldwell, R. M. 1937. *Rhynchosporium* scald of barley, rye and other grasses. J. Agric. Res. 55:175-198.
4. Conners, I. L. 1967. An annotated index of plant diseases in Canada. Canada Dept. Agric. Pub. 1251. Queen's Printer and Controller of Stationery, Ottawa, Canada.
5. Farr, D. F., Bills, G. F., Chamuris, C. P., and Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. American Phytopathological Society, St. Paul, MN.
6. Jackson, L. F., and Webster, R. K. 1976. Race differentiation, distribution, and frequency of *Rhynchosporium secalis* in California. Phytopathology 66:719-725.
7. Larter, E. N. 1976. Triticale. Pages 117-120 in: Evolution of Crop Plants. W. Simmonds, ed. Longman Scientific and Technical, Essex, England.
8. Owen, H. 1958. Physiologic specialization in *Rhynchosporium secalis*. Trans. Br. Mycol. Soc. 41:99-198.
9. Owen, H. 1973. *Rhynchosporium secalis*. No. 387 in: Descriptions of Pathogenic Fungi and Bacteria. Commonw. Mycol. Inst., Kew, England.
10. Perez-Fernandez, J., and Welty, R. E. 1991. Histopathology of orchardgrass infection by *Rhynchosporium orthosporium*. Mycologia 83: 774-778.
11. Saari, E .E., Varughese, G., and Abdalla, O. A. 1986. Triticale diseases: Distribution and importance. Pages 208-231 in: Proc. Int. Triticale Symp., Aust. Inst. Agric. Sci., Occasional Pub. No. 24, Sydney, Australia.
12. Skajennikoff, M., and Rapilly, F. 1985. Consequences of triticale introduction on the parasitic fungi of wheat. Pages 537-549 in: Proc. Genet. Breeding Triticale, 3rd. EUCARPIA, INRA.