

Etiology of *Rhizoctonia cerealis* in Sharp Eyespot of Wheat

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## ABSTRACT

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Isolates of a *Rhizoctonia* sp. with binucleate hyphal cells were obtained from sharp eyespot lesions on wheat culms in Ohio. These isolates were compared to known isolates of *Rhizoctonia cerealis* with reference to cultural morphology, growth rate, hyphal anastomosis, and pathogenicity on wheat. The wheat isolates were similar in cultural morphology and anastomosed with the *Ceratobasidium*-anastomosis-group 1 (CAG 1) tester isolate of *R. cerealis*. Growth rates at 23 C on freshly prepared potato-dextrose agar ranged 10.5–11.7 mm/24 hr for the wheat isolates and 7.2–11.0 mm/24 hr for the known isolates of *R. cerealis*. Inoculated plants

developed sharp eyespot lesions on culms and white head symptoms typical of the disease. Virulence ratings for the wheat isolates on inoculated wheat seedlings ranged 2.0–4.4 on a scale of 0 = no symptoms and 5 = dead plants. The rating for the CAG 1 tester isolate (*R. cerealis*) was 4.0. Representative isolates from CAG 2 through 7 were nonpathogenic (0.0–0.2 ratings). None of the wheat isolates tested caused root rot on wheat seedlings. Based on these results, the pathogenic binucleate isolates from wheat were assigned to *R. cerealis*.

Sharp eyespot is a common disease of wheat (*Triticum aestivum* L.) in the temperate regions of the world (1–3, 7, 8, 15, 16, 20–22). The disease generally causes minimal economic loss (8) and frequently occurs in association with more damaging root and stem diseases (22, 23).

In North America, the first report of sharp eyespot on wheat was by Sprague in 1934 (20). Sprague (21) later attributed the cause to a *Rhizoctonia* sp. Based on morphological characters, Blair (1) identified the fungus isolated from sharp eyespot lesions on wheat stems from Canada and England as *Rhizoctonia solani* Kuhn. Subsequent work in England (7, 8, 15) and in the United States (3) supported Blair's identification of *R. solani* as the causal agent of sharp eyespot. Recently, Stern and Jones (22) identified the cause of sharp eyespot as *R. solani* and assigned it to the hyphal anastomosis group 4 (AG 4), as erected by Parmeter et al (13). Stern and Jones (22) reported that only AG 4 isolates of *R. solani* caused damping-off and sharp eyespot lesions on wheat. In 1977, Van der Hoeven (2) described a new species, *R. cerealis* Van der Hoeven, causing sharp eyespot lesions on wheat in the Netherlands. *R. cerealis* differs from *R. solani*, in having predominantly binucleate hyphal cells and a relatively slow growth rate. Thus, two different fungi, resembling one another in morphological features, cause similar symptoms on small grains.

Groupings based on hyphal anastomosis among isolates having common biological affinities has greatly facilitated the identification of *R. solani* (13, 14). Burpee et al (5) observed hyphal anastomosis among isolates of *Ceratobasidium cornigerum* (Bourd.) Rogers and related fungi having *Rhizoctonia* imperfect states. Based on hyphal pairings, seven *Ceratobasidium* anastomosis groups (CAG) were established. Within each CAG, little homogeneity occurred among isolates with respect to host, except for CAG 1 isolates, which were associated with members of the Gramineae (5). Burpee later found that isolates assigned to CAG 1 anastomosed with the type culture of *R. cerealis*, demonstrating that isolates of *R. cerealis* comprise a common anastomosis group, CAG 1 (4). He reported that isolates of *R. cerealis* from the United States and elsewhere were the cause of yellow patch of turfgrass (4). *R. cerealis* has been reported as the

cause of sharp eyespot in the Netherlands (2, 23), Germany (16), and South Africa (19).

Plants with white heads and sharp eyespot lesions on the base of culms were found in several locations during a survey of wheat fields in Ohio. Fungi resembling *R. solani* were isolated from these lesions. However, these isolates grew slower and had narrower hyphae than typical isolates of *R. solani* (14).

The purpose of this study was to identify the isolates obtained from sharp eyespot lesions and to determine their pathogenicity on wheat in Ohio. A preliminary report (11) has been published.

## MATERIALS AND METHODS

**Isolation and sources.** In May, June, and July 1980, wheat culms with sharp eyespot lesions were collected from commercial fields and experimental plots in Champaign, Clark, Licking, Pickaway, Wayne, and Wood counties in Ohio. Culm sections, 3–5 mm long, containing lesions were surface sterilized in a mixture of 5.25% sodium hypochlorite and distilled water (1:1, v/v) for 30 sec and then pressed between paper towels to remove excess moisture. Sections were placed on 2% water agar (WA) containing 300 µg/ml of streptomycin sulfate and incubated at 23–25 C. Hyphal tips were transferred to Difco (Difco Laboratories, Detroit, MI 48232) potato-dextrose agar (DPDA), or DPDA supplemented with 1 g yeast extract (PDAYE) per liter.

CAG tester isolates, obtained from Lee Burpee (University of Guelph, Guelph, Ontario, Canada), included ATCC 44233 (CAG 1), ATCC 34969 (CAG 2), BN 31 (CAG 3), BN 38 (CAG 4), BN 37 (CAG 5), ATCC 13247 (CAG 6), ATCC 13244 (CAG 6), and FTCC 585 (CAG 7). Two isolates of *R. cerealis*, CBS 236.77, the type culture, and CBS 560.77, were obtained from Centraalbureau voor Schimmelcultures, Baarn, the Netherlands. An isolate (W 101) of *R. solani* AG 4 from *Chenopodium album* L. from Ohio was used for comparison.

**Nuclear staining.** Cultures on DPDA were stained by a rapid staining technique with 0.5% aniline blue and by a HCl-Giemsa nuclear staining procedure (9), which allowed counting of nuclei in vegetative cells.

**Hyphal anastomosis.** Hyphal anastomosis was observed on agar-coated slides as previously described (10). All wheat culm isolates were paired with known CAG tester isolates to determine their affinities. Isolates of *R. cerealis* (CBS 236.77, CBS 560.77, and ATCC 44233) and a representative isolate (3.80) from diseased wheat culms were paired in all possible combinations.

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**Growth rate and cultural characteristics.** Agar disks (7 mm in diameter) cut from the margin of actively growing colonies on DPDA were transferred to 15 × 100-mm plastic petri dishes containing 15 ml of potato-dextrose agar freshly prepared from potatoes (FPDA) (6). Three dishes of each isolate were incubated at 23 C in the dark. Two measurements at right angles were taken and the increase in colony diameter between 24 and 48 hr of growth was recorded. Cultural morphology and color were compared during the first week of growth and after 23 days.

**Pathogenicity.** Oat kernel inoculum was prepared by autoclaving 100 ml of whole oat kernels and 50 ml of distilled water in a 250-ml Erlenmeyer flask for 1 hr on two consecutive days. Colonized agar disks of each test isolate were transferred to flasks and incubated at 23–26 C for 10–14 days prior to use.

Seeds of spring wheat, cultivar 'Era,' were surface sterilized in a mixture of 5.25% sodium hypochlorite and 95% ethyl alcohol (1:1, v/v) for 30 sec, then rinsed in sterile water and planted wet in autoclaved Wooster silt loam-sand mixture (1:1, v/v). Five seeds were planted 2.5 cm deep in the soil-sand mixture in a 15-cm-diameter plastic pot. Five replicate pots were used per isolate. Plants were maintained in the greenhouse at 18–27 C with 10 hr of supplemental light (1,050 lux) per day.

Isolates 3.80, 14.80, and 31.80 were tested for pathogenicity on wheat plants in the greenhouse. Oat kernels infested with one of the isolates were autoclaved and used for the control. Seedlings were inoculated by placing one infested oat kernel 1 cm below the soil surface in contact with the coleoptile as it emerged from the soil. At the milky dough stage of development, plants were examined for premature death of tillers and the presence of sharp eyespot lesions on culms. Koch's postulates were fulfilled by reisolation of fungi from culms with lesions and retesting for pathogenicity.

A seedling assay was used to evaluate the pathogenicity and virulence of all wheat isolates and the CAG tester isolates. Seven wheat seeds were planted 2.5 cm deep in 500-ml styrofoam cups (Dart 16MJ20, Dart Container Corporation, Mason, MI 48854) containing 375 ml of autoclaved soil-sand mixture. Five replicate cups per isolate were randomized in a growth chamber at 10 ± 1 C with 10 hr of light (intensity 2,430 lux) per day. Emerging seedlings were inoculated by placing an infested oat kernel 1 cm below the soil surface in contact with the coleoptile. Oat kernels infested with one of the isolates were autoclaved and used for the control. After 28–34 days, plants were washed free of adhering soil and rated for lesion development. Disease severity ratings were based on a modification of the system used by Pitt (15). Plants were rated on a 0–5 scale: 0 = no symptoms, 1 = browning of the outer leaf sheath, 2 = definite eyespot lesion on outer leaf sheath less than 5 mm in length, 3 = one to several eyespot lesions on outer leaf sheath greater than 5 mm in length, 4 = lesion with penetration to inner leaf sheath(s), and 5 = lesion with penetration to center of culm or dead plant. The test was repeated.

The ability of wheat isolates to cause root rot of wheat seedlings was tested by using an adaptation of Schmitthenner and Hilty's (18) inoculum layer technique. Styrofoam cups, described above, were filled with 300 ml of autoclaved soil-sand mix. A completely colonized WA layer of inoculum from a petri dish culture was placed to cover the top of the soil. A noncolonized agar layer was used for the control. Seven surface-sterilized wheat seeds were arranged on top of the agar inoculum and then covered with 50 ml of the soil-sand mix. Five replicate pots were randomized on the greenhouse bench and plants were maintained at 10–25 C with supplemental light (1,150 lux) for 28 days. Seedlings were washed free of adhering soil, and root length and fresh weight of tops were recorded. The test was repeated.

## RESULTS

**Nuclear staining.** All 28 isolates from wheat culms with sharp eyespot lesions had binucleate hyphal cells. Both the rapid-staining technique with 0.5% aniline blue and the HCl-Giemsa nuclear stain were effective for determining the nuclear condition of all isolates.

**Hyphal anastomosis.** Vegetative hyphae of the 24 wheat isolates from Wayne, Wood, Clark, Licking, and Champaign counties

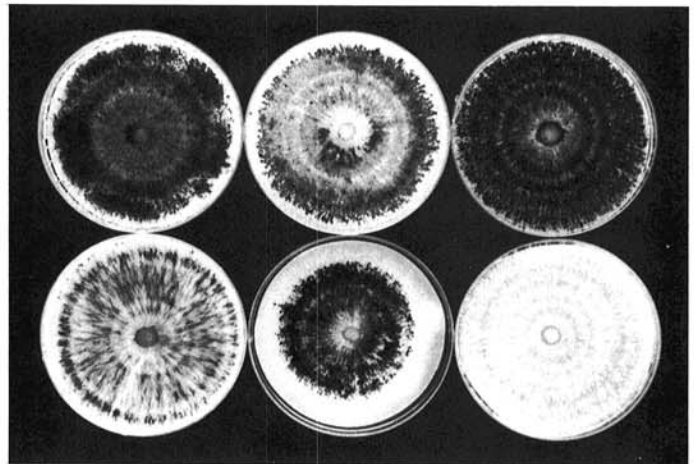
anastomosed with the CAG 1 tester isolate (ATCC 44233) of *R. cerealis*. The four wheat isolates from Pickaway County failed to anastomose with the CAG 1 tester or with the other CAG 2 through 7 tester isolates. Hyphae of the wheat isolate (3.80), the CAG 1 tester isolate, the type culture of *R. cerealis* (CBS 236.77) and another *R. cerealis* isolate (CBS 560.77) fused with one another, confirming common anastomosis affinities among isolates tested.

**Cultural characteristics.** All 28 binucleate isolates of *Rhizoctonia* obtained from wheat culms with sharp eyespot lesions formed yellow-white to light-tan colored mycelium on FPDA during the first week of growth. In most cultures, mycelial pigmentation increased with age, resulting in light-tan to dark-brown coloration after 23 days of growth (Fig. 1). Sclerotial development varied greatly among isolates with some producing no or very few sclerotia, and others having many darkly pigmented sclerotia covering the agar surface. The four isolates from Pickaway County, which failed to anastomose with CAG 1 through 7, produced no sclerotia and hyphae remained yellow-white during the 23 days of this study. Hyphae of all wheat isolates studied ranged from 3.0–7.5 μm in diameter.

**Growth rates.** The growth rate of the CAG 1 tester isolate (ATCC 44233) (11 mm/24 hr) was within the range of growth rates of the wheat isolates that were assigned to CAG 1 (10.7–11.5 mm/24 hr), but the type culture of *R. cerealis* (CBS 236.77) grew slower (7.2 mm/24 hr) than the other fungi tested (Table 1). A wheat isolate (18.80) from Pickaway County, which failed to anastomose with tester isolates from CAG 1 through 7, grew slightly faster than the CAG 1 isolates (14.5 mm/24 hr). The AG 4 isolate of *R. solani* grew three times as fast (30 mm/24 hr) as the wheat isolates assigned to CAG 1 and the two *R. cerealis* isolates.

**Pathogenicity.** The three wheat isolates (3.80, 14.80, and 31.80) caused typical sharp eyespot lesions on the base of wheat culms (Fig. 2). One to several tillers per plant were killed prematurely due to the development of sharp eyespot lesions. Death of tillers caused the head to lose color, resulting in the white-head symptom. Lesions and white heads did not develop on control plants. After isolation of fungi from lesions and reinoculation onto seedlings, sharp eyespot lesions and white head symptoms were again produced on plants.

Variation was found in the virulence of binucleate isolates of *Rhizoctonia* using the wheat seedling assay (Table 2). Wheat isolates that anastomosed with the CAG 1 tester isolate produced sharp eyespot lesions on wheat seedlings, but significant differences in the severity of disease occurred. The CAG 1 tester isolate was pathogenic, but the other CAG tester isolates (CAG 2 through 7) and the binucleate of *Rhizoctonia* isolates from Pickaway County,



**Fig. 1.** Cultural morphology of *Rhizoctonia cerealis* isolates grown on potato-dextrose agar (freshly prepared from potatoes) for 23 days at 23 C in the dark. Top row (L to R): type culture CBS 236.77; wheat isolates from Ohio 1.80 and 14.80; and bottom row (L to R): *Ceratobasidium* anastomosis-group I tester isolate ATCC 44233; wheat isolates from Ohio 3.80 and 23.80.

which failed to anastomose with CAG 1 through 7, were nonpathogenic, causing only slight browning on some leaf sheaths.

None of the 28 wheat isolates caused visible root rot, root stunting, or reduction of top growth of seedlings when roots were allowed to grow through an agar layer colonized by the test fungi. Some of the isolates assigned to CAG 1 caused sharp eyespot lesions on leaf sheaths of a few plants, but no root damage was noted.

TABLE 1. Growth rates<sup>a</sup> of binucleate *Rhizoctonia* isolates from wheat, *R. cerealis* isolates, and a *R. solani* AG 4 isolate

Isolate	Anastomosis group	Growth rate (mm/24 hr)
1.80	CAG 1	11.5
3.80	CAG 1	10.5
14.80	CAG 1	11.7
23.80	CAG 1	10.7
ATCC 44233 <sup>b</sup>	CAG 1	11.0
CBS 236.77 <sup>c</sup>	CAG 1	7.2
18.80 <sup>d</sup>	BN	14.5
W101 <sup>e</sup>	AG 4	30.0

<sup>a</sup>Incubated at 23 C on potato-dextrose agar freshly prepared from potatoes.

<sup>b</sup>CAG 1 tester isolate of *R. cerealis*.

<sup>c</sup>Type culture of *R. cerealis*.

<sup>d</sup>BN = Binucleate *Rhizoctonia* isolate from Pickaway Co. that did not anastomose with CAG 1 through 7.

<sup>e</sup>Isolate of *R. solani* AG 4.

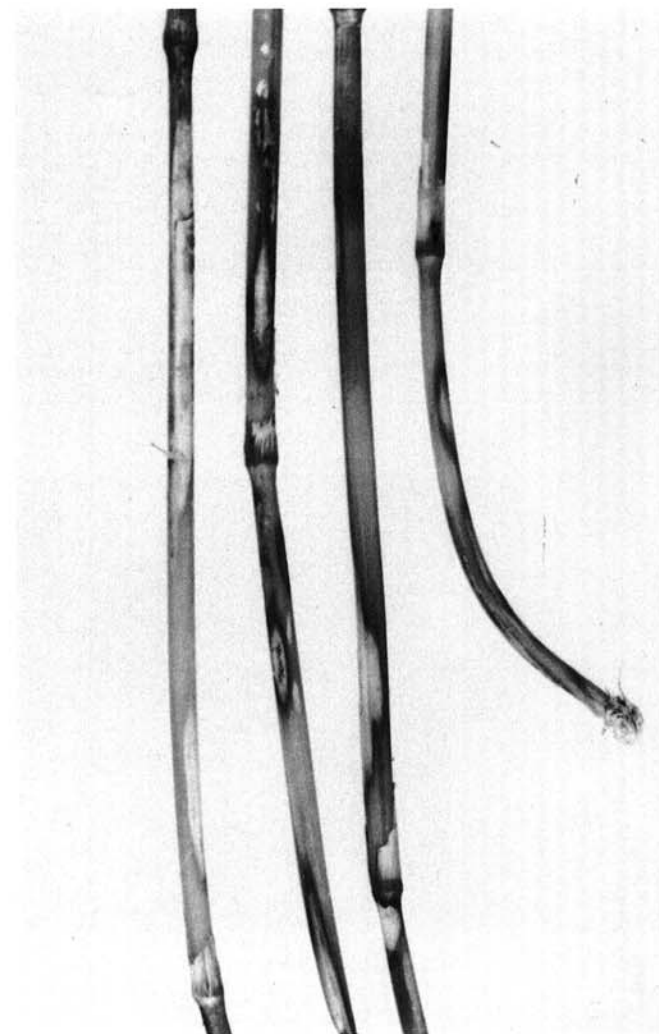


Fig. 2. Sharp eyespot lesions on the culm bases of cultivar Era spring wheat inoculated with the isolate 3.80 of *Rhizoctonia cerealis* from Ohio.

## DISCUSSION

The results of this study indicate that the pathogenic binucleate isolates of *Rhizoctonia* obtained from wheat culms with sharp eyespot lesions were *R. cerealis* as described by Van der Hoeven (2). Cultural morphology of the wheat isolates paralleled those reported previously for *R. cerealis* (2,4), but more variation in color was detected. Considerable variation in sclerotial production occurred among different isolates of *R. cerealis* from wheat. Burpee (4) also found sclerotial production to be of limited taxonomic value. Growth rate of the CAG 1 tester isolate was similar to that of the isolates from wheat, but the type culture (CBS 236.77) grew slower than the other isolates tested. The one isolate tested, which failed to anastomose with CAG 1 through 7, grew somewhat faster than the *R. cerealis* isolates. These results indicate that growth rate may facilitate separation of *R. cerealis* from other binucleate isolates.

Burpee suggested that hyphal anastomosis and host specificity would be valuable characters for identifying *R. cerealis* (4). In an anastomosis test, hyphal fusion occurred between isolates of *R. cerealis* paired in all possible combinations regardless of their origin. When tested for pathogenicity, only isolates that anastomosed with CAG 1 were pathogenic on wheat seedlings

TABLE 2. Virulence of binucleate *Rhizoctonia* isolates obtained from wheat and *Ceratobasidium* anastomosis-group (CAG) tester isolates on wheat seedlings

Isolate	Anastomosis group	Disease rating <sup>w</sup>
14.80	CAG 1	4.4 a <sup>y</sup>
5.80	CAG 1	4.3 a
23.80	CAG 1	4.3 a
13.80	CAG 1	4.2 a
1.80	CAG 1	4.1 ab
34.80	CAG 1	4.0 ab
22.80	CAG 1	4.0 ab
ATCC 44233 <sup>x</sup>	CAG 1	4.0 ab
29.80	CAG 1	3.9 abc
41.80	CAG 1	3.9 abc
8.80	CAG 1	3.8 abcd
3.80	CAG 1	3.7 abcd
4.80	CAG 1	3.7 abcd
12.80	CAG 1	3.7 abcd
35.80	CAG 1	3.7 abcd
43.80	CAG 1	3.7 abcd
26.80	CAG 1	3.6 abcd
42.80	CAG 1	3.6 abcd
33.80	CAG 1	3.6 bcd
40.80	CAG 1	3.4 bcd
39.80	CAG 1	3.4 bcd
24.80	CAG 1	3.3 cd
32.80	CAG 1	3.2 d
31.80	CAG 1	3.1 d
27.80	CAG 1	3.1 d
25.80	CAG 1	2.0 e
17.80	BN <sup>z</sup>	0.3 f
20.80	BN	0.3 f
16.80	BN	0.2 f
ATCC 13247 <sup>x</sup>	CAG 6	0.2 f
ATCC 34969 <sup>x</sup>	CAG 2	0.2 f
ATCC 13244 <sup>x</sup>	CAG 6	0.1 f
BN 31 <sup>x</sup>	CAG 3	0.1 f
BN 37 <sup>x</sup>	CAG 5	0.1 f
FTCC 585 <sup>x</sup>	CAG 7	0.1 f
BN 38 <sup>x</sup>	CAG 4	0.1 f
18.80	BN	0.0 f
Control		0.0 f
LSD ( $P = 0.05$ )		0.6

<sup>w</sup>Disease rating based on a 0-5 scale (0 = no symptoms, 5 = dead).

<sup>x</sup>CAG tester isolates.

<sup>y</sup>Means followed by the same letter are not significantly different ( $P = 0.05$ ) according to Duncan's new multiple range test.

<sup>z</sup>BN = binucleate *Rhizoctonia* isolates that failed to anastomose with CAG 1 through 7.



(Table 2). Our findings also support the conclusion of Burpee (4) that isolates of *R. cerealis* comprise a common anastomosis group, CAG 1.

The isolates from Pickaway County (isolates 16.80, 17.80, 18.80, and 20.80) were obtained from wheat culms with eyespot lesions, but when these were inoculated onto seedlings no symptoms developed (Table 2). It was possible that these fungi were present on the diseased culms together with *R. cerealis*, but the isolation technique may have favored the growth of these nonpathogenic isolates instead of *R. cerealis*. Because none of these isolates anastomosed with any of the CAG tester isolates, more work is needed to identify them.

Stern and Jones (22) reported that nine of 10 isolates obtained from wheat culms anastomosed with an AG 4 tester isolate of *R. solani* and that pathogenicity trials proved that the *R. solani* AG 4 isolates caused sharp eyespot lesions on wheat plants grown in the greenhouse (22). These results with *R. solani* and those of the present and other investigations (15,23) with *R. cerealis* indicate that two different fungi cause sharp eyespot lesions on cereals. Based on these studies it is recommended that nuclear staining and observations of hyphal anastomosis be used as methods for differentiating isolates of *Rhizoctonia* spp. from cereals (13,14).

Considerable variation in the ability of isolates to parasitize roots and culms of cereals has been reported (1,3,15,17,22). Blair (1) observed that isolates, identified as *R. solani*, produced two distinct types of injury on wheat. Isolates from England caused severe root rot, whereas those from Canada attacked only the lower culm of wheat plants (1). The results of our study and reports by Bruehl (3), Pitt (15), and Stern and Jones (22) indicate that isolates from sharp eyespot lesions do not attack roots. Samuel and Garrett (17) reported a severe root rot of wheat in South Australia caused by *R. solani* and noted that although roots were severely rotted, culms were never affected. Recently, Murray (12) reported that isolates of *R. solani* AG 3 from Scotland attacked roots of barley, causing a stunt disease. In view of reports of *R. solani* attacking root and culms of cereals and the relatively recent use of nuclear staining and hyphal anastomosis as an aid in differentiating isolates and species of *Rhizoctonia* (*R. cerealis* and *R. solani*), a comparative study is needed to determine which isolates or species attack roots or culms or both.

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