A Semiselective and Diagnostic Medium for *Gaeumannomyces graminis* var. *tritici*

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This paper is dedicated to the memory of B. Ben Bohlool, who died tragically in 1991. He will long be remembered as an eminent soil microbiologist and teacher as well as a great gentleman with a keen sense of humor and enthusiasm for life.
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ABSTRACT

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Isolation of *Gaeumannomyces graminis* var. *tritici*, causal agent of take-all, from wheat tissues often is complicated by the presence of *Pythium* spp., *Rhizoctonia* spp., *Fusarium* spp. or secondary fungal colonists. Because *G. g. tritici* does not typically sporulate in culture, identification of putative isolates is based on cultural characteristics, pathogenicity tests, and production of perithecia on infected tissue. A semiselective medium designated R-PDA was developed that aids in the isolation and identification of *G. g. tritici*. The medium consists of dilute potato-dextrose agar amended with 100 μg ml^{-1} rifampicin and 10 μg ml^{-1} tolclofos-methyl. Identification of *G. g. tritici* is aided based on its ability to alter the color of rifampicin in R-PDA from orange to purple. This reaction occurs in as little as 24 h. R-PDA was more effective in isolating putative isolates of *G. g. tritici* from wheat with symptoms of take-all than was SM-GGT3, another semiselective medium for *G. graminis* var. *tritici*.

Additional keywords: barley, *Triticum aestivum* L., *Hordeum vulgare* L., 25-desacetylrifampicin.

Take-all, caused by *Gaeumannomyces graminis* (Sacc.) Arx & D. Olivier var. *tritici* J. Walker, is probably the most important crown and root rot disease of wheat (*Triticum aestivum* L.) worldwide (2). *G. g. tritici* also is a pathogen of barley (*Hordeum vulgare* L.). The pathogen survives between crops in infested plant debris, volunteer cereals, or graminaceous weeds. The pathogen infects young roots and causes dark brown to black lesions that later coalesce. The fungus may rot the entire crown and root system and advance up the base of the tillers. Symptoms of take-all also include generalized chlorosis, stunting, sterile grain heads (white heads), and premature plant mortality (2,6). Incidence and severity of take-all is greatest when wheat or barley is cropped intensively in reduced-tillage systems and under irrigation or high rainfall (6,23).

The isolation and identification of *G. g. tritici* from diseased tissue can be difficult. For example, in the Pacific Northwest, wheat roots infected with *G. g. tritici* also commonly harbor *Pythium* spp. (4,19), *Fusarium* spp. (6), and *Rhizoctonia* spp. (6,25), which can interfere with the recovery of the take-all pathogen. Furthermore, take-all lesions caused by *G. g. tritici* are rapidly invaded by many opportunistic soil fungi that eventually displace the pathogen (14).

Because *G. g. tritici* typically does not sporulate in culture, routine identification must rely on cultural characteristics such as pigmentation, thick runner hyphae, and hyphal curling at the colony margins (1,7). To confirm identification, time-consuming pathogenicity tests must be conducted so that perithecia are produced on wheat. Recently, DNA probes, polymerase chain reaction, and restriction fragment length polymorphisms have been used to detect *G. g. tritici* and related fungi in host tissue (15–17,24,32).

Juhnke et al (20) reported a semiselective medium for *G. g. tritici* designated SM-GGT3; since then two modifications of this medium, SM-GGT4 (10) and SM-GGT7 (11), that are as effective as SM-GGT3, have been reported. SM-GGT3 is commercial potato-dextrose agar amended with L-β-3,4-dihydroxyphenylalanine (L-DOPA), which is probably utilized by *G. g. tritici* and selected other fungi for melanin biosynthesis, dicloran for inhibition of *Rhizopus* spp., 1-(3,5-dichlorophenyl)-3-methoxymethyl-pyrrolidin-2,4-dion (Hoe 00703) for inhibition of *Fusarium* spp., metalaxyl for inhibition of *Pythium* spp., and streptomycin for inhibition of bacteria. Limitations of SM-GGT3 include a high rate of bacterial contamination and a low rate of recovery of *G. g. tritici* from infected wheat tissues.

During routine culturing of *G. g. tritici* on homemade potato-dextrose agar amended with rifampicin, which is inhibitory to a broad spectrum of bacteria, we noticed that the color of the medium around the fungal colony changed from orange to purple. This reaction in the medium appeared to be specific for *G. g. tritici*. The purpose of our study was to determine whether this reaction could be exploited as an aid in the isolation and identification of *G. g. tritici*.

A preliminary report of portions of this work was previously published (9).

MATERIALS AND METHODS

Media. Dilute potato-dextrose agar (dPDA) consisted of the filtered extract from 40 g of peeled, boiled potatoes increased to a volume of 1 L with deionized water, 4 g of dextrose and

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TABLE 1. Fungi and sources

Fungus	Anastomosis group	Isolate	Origin[z] (host, location)	Source
Aspergillus spp.			Wheat, Washington	This work
Bipolaris spp.			Barley, Washington	This work
Cephalosporium gramineum			Wheat, Washington	T. Murray (Wash. State Univ.)
Fusarium culmorum				This work
F. equiseti			Pea, Washington	W. Kaiser (USDA-ARS, Pullman)
F. oxysporum f. sp. *pisi* race 2				
F. sambucium			Potato, Washington	
F. solani			Pea, Washington	
Gaeumannomyces cylindrosporus		WF912	Corn, Illinois	H. Wilkinson (U. of Illinois)
G. graminis		AB	Annual bluegrass, Indiana	D. Huber (Purdue Univ.)
G. graminis var. *avenae*		1825	*Agrostis* spp., Rhode Is.	J. Henson (Montana State Univ.)
		1840	Oat, United Kingdom	
		2505	*Agrostis* spp., Australia	
		2506	*Agrostis* spp., Colorado	
G. graminis var. *graminis*		TX1	Rice, Texas	C. Gonzalez (Texas A & M)
		502.1L	-, Georgia	D. Huber
		WF911	-, Illinois	H. Wilkinson
G. graminis var. *tritici*		WF861	Wheat, Illinois	
		WF913		
		K1	Wheat, Switzerland	G. Défago (ETH, Zürich)
		11	Wheat, Indiana	D. Huber
		8	Wheat, South Carolina	
		1817	Wheat, France	J. Henson
		1818	*Agrostis* spp., France	
		1845; 1847	-, Australia	
		1851; 1852	Wheat, Poland	
		1856; 1857	-, Germany	
		1865	Wheat, Denmark	
		1866	Rye, Denmark	
		1801; 1804	-, United Kingdom	
		516; 541	-, Arkansas	E. A. Milus (Univ. of Arkansas)
		WX	-, South Carolina	
		602.4, 211.1	-, Georgia	
		Hollinsville		
		L102; L103	Wheat, Washington	R. J. Cook (USDA-ARS, Pullman)
		L103hv		
		L104; L105		
		L106; L108		
		L108hv		
		L109; L110		
		L111; L112		
		L113; L114		
		L116; L117		
		L117hv		
		L118; L119		
		MV101; MV102		
		MV103; MV104		
		MV105; MV106		
		MV107; MV108		
		MV109; MV110		
		MV111; MV112		
		MV113; MV114		
		MV115; MV116		
		MV117; MV118		
		MV119; MV120		
		MV120hv		
		MV121; MV122		
		MV123; MV124		
		MV125; MV126		
		MV127; MV135		
		MV128; P1; P6		
		P9; P10; P11		
		P15; P18		
		PIT; GEM II		
		R3-111a-1		
		R3-111a-1hv		
		SCS4; SCS6		
		MI-88	-, Missouri	W. Bockus (Kansas State Univ.)
		JO-1; HV-3	-, Kansas	
		RL-1; RL-4		
		SA-1; STV-1		
		GH-87; GH-90		
		PL		
		Pend1	Wheat, Oregon	This work
G. incrustans		WF914	Corn, Illinois	H. Wilkinson
Bipolaris spp.			Wheat, Washington	This work

(*continued on next page*)

[z] Hyphen (-) indicates that this information is unavailable.

TABLE 1. (*continued from preceding page*)

Fungus	Anastomosis group	Isolate	Origin[z] (host, location)	Source
Leptosphaeria korrae		CD18-103	Turf grass, Washington	G. Chastagner (Wash. State Univ.)
		R-49, TC1-19		
Mucor spp.			Soil, Washington	This work
Penicillium spp.			Wheat, Washington	
Pseudocercosporella herpotrichoides		Ph89-25-1b	Wheat, Washington	T. Murray
Pythium dissotocum		ATCC 34994	-, -	R. J. Cook
P. graminicola		ATCC 34999	-, -	
P. heterothallicum		ATCC 18198	-, -	
P. intermedium		ATCC 36445	-, -	
P. irregulare		ATCC 11120	-, -	
P. macrosporum		CBS 574.80	-, -	
P. paroecandrum		ATCC 16971	-, -	
P. rostratum		ATCC 16972	-, -	
P. sylvaticum		ATCC 18196	-, -	
P. torulosum			-, -	
P. ultimum var. *sporangiiferum*			-, -	
P. ultimum var. *ultimum*			-, -	
P. volutum			-, -	
Pyrenophora semeniperda (*Drechslera* state)			Wheat, Washington	This work
Binucleate *Rhizoctonia* spp.	AG-A	SN1	-, -	R. J. Cook
	AG-B	C302	-, -	
	AG-Ba	B7	-, -	
		C460; C484	-, -	
	AG-Bb	C157; C344	-, -	
	AG-C	STC1	-, -	
	AG-D	C60; C73	-, -	
		10B7	-, -	
		23s-2; 23s-3	Wheat, Oregon	
		23s-4	-, -	
		742; 743	Wheat, Washington	
	AG-E	F18; RH15	-, -	
		TM1-1	-, -	
		116; 118	Wheat, Washington	
		11b-9		
		16b-1	Barley, Washington	
	AG-G	AHC9; Fa479	-, -	
	AG-I	AV2; Cre3a	-, -	
		PT5	-, -	
	AG-K	AC1; SH10	-, -	
		55-D33	-, -	
		11-2w1-2	Barley, Washington	
		625; 15b-1	Wheat, Washington	
		256; 25b-3	Alfalfa, Oregon	
		25b-5		
	AG-Q	Rk-3	-, -	
R. oryzae	AG-WAG-O	35b-1; 39b-1	Barley, Washington	
		43b-9; 435		
		437		
		241; 371	Wheat, Washington	
		521; 541		
		552		
R. solani	AG-4	36b-2	Wild grass, Oregon	
		632; 635	Wheat, Washington	
		637; 63b-1		
	AG-5	D1; D23	Wheat, Oregon	
	AG-6HGI	UBU-14	-, -	
	AG-6GV	SO-21	-, -	
	AG-Ma	45b-6	Wheat, Idaho	
	AG-X1	53b-1	Barley, Washington	
		210b-2	Barley, Oregon	
		210b-3		
		42b-2	Wheat, Idaho	
	AG-8	FAC-1727-B	-, -	
		S1-BS1	-, -	
		C-1; 713-s1	Barley, Washington	
		713-s2; 722		
		31b-1	Barley, Oregon	
		14b-1; 29b-6	Wheat, Washington	
		372; 561		
		562; 563		
		45b-1		
Rhizopus spp.			Soil Washington	This work
Trichoderma spp.			Wheat, Washington	T. Murray
Trichoderma koningii		7a; 7c	Wheat, Australia	A. Simon (CSIRO, Australia)
		AST-1		

18 g of agar (Sigma Chemical Co., St. Louis, MO). The medium was adjusted to pH 6-6.5 when necessary with concentrated HCl or NaOH after autoclaving. Rifampicin and tolclofos-methyl were added after autoclaving when the media cooled to below 60 C. To prepare R-PDA, rifampicin (100 $\mu g\ ml^{-1}$) (Sigma Chemical Co.) and tolclofos-methyl (10 $\mu g\ ml^{-1}$) (Rizolex 75 WDG, Sumitomo Co., Japan) were added to autoclaved dPDA. SM-GGT3 was prepared as previously described (20).

Media were stored at 4 C in the dark and used 1-5 days after preparation. When stored for 2-5 days at temperatures over 27 C or in the light, the color of rifampicin in R-PDA often changed from orange to brownish purple, precluding observation of the reaction. The color of L-DOPA in SM-GGT3 also was sensitive to temperature and light, changing color from clear to brown.

Fungal strains. Table 1 provides a list of the fungi used in this study. The 96 isolates of *G. g. tritici* were from North America, Europe, and Australia. A total of 106 other fungi, many isolated from the wheat rhizosphere, also were used. Fungi were stored on slants of dPDA at 4 C in the dark. Inocula for experiments were grown on plates of dPDA at room temperature (21-24 C) in the light for 7-10 days before use. Agar plugs generally were taken from the margins of actively growing cultures.

Color change of rifampicin. The ability of the fungi to alter the color of rifampicin from orange to purple was tested on dPDA amended with rifampicin (100 $\mu g\ ml^{-1}$). Agar plugs (9-mm diameter) were inverted on the surface of the medium in the center of a petri dish. Dishes were incubated at room temperature in the dark, and the color of the media surrounding the inoculum plug and mycelia was observed after 24, 48, and 72 h. Treatments were replicated at least three times within an experiment, and each strain was tested at least twice.

Fungal inhibition in vitro. Agar plugs (4-mm diameter) of the fungi were placed on the surface of dPDA amended with rifampicin and tolclofos-methyl or SM-GGT3 in petri dishes as described above. Nonamended dPDA served as the control. Dishes were incubated in the dark at 21-24 C. Colony size was expressed as the average of two radii measured in opposite directions. Growth inhibition on amended media was expressed as the percent suppression of radial length compared with mycelia on dPDA.

Growth of *G. g. tritici* and *Rhizoctonia* spp. on R-PDA and SM-GGT3. The first experiment compared the growth on R-PDA and SM-GGT3 of six isolates of *G. g. tritici* and six isolates of *Rhizoctonia* spp.; the treatment design was a 2 media × 12 fungi factorial in a randomized complete block design. Radial length and color changes in the media were measured at 11 days after inoculation. The second experiment compared the growth on R-PDA and SM-GGT3 of 22 isolates of *R. solani* from wheat and barley; the treatment design was a 2 media × 22 strain factorial in a randomized complete block design. The radius of each colony was measured at 4, 8, and 16 days, but only the data from day 16 is shown. In both experiments, treatments were replicated three times, and each experiment was repeated with similar results. The main effects and interactions were analyzed for significance by SAS general linear model procedures (29-31). In both experiments isolate × media interactions were highly significant ($P =$ 0.0001), thus the mean growth of all isolates was compared only within a medium and comparisons between R-PDA and SM-GGT3 were made only within a single isolate.

Isolation of *G. g. tritici* from wheat. The efficacy and efficiency of isolating *G. g. tritici* from wheat tissues was evaluated on R-PDA and SM-GGT3. Wheat plants (past the heading stage) with symptoms of take-all were collected from commercial wheat fields near Pullman and Mount Vernon, WA, and Pendleton, OR. The Pullman sample was brought in for diagnosis and was processed within 24 h of collection. The Mount Vernon sample was collected and stored in the dark at 8 C for ~2 mo before it was processed. The Pendleton sample was from wheat stubble that had been burned and the soil disked several days earlier. For all samples, soil was shaken from the roots, and stems were cut 4 cm above the crown immediately before they were processed. Plants were either washed for several minutes under running water, surface sterilized by immersion in 1% silver nitrate for 30 s, and finally rinsed for 30 s in sterile deionized water as described by Juhnke et al (20); or they were washed for 60 min under running deionized water and then rinsed twice in sterile deionized water for 30 s. Tissue was blotted dry on filter paper before transfer to media in petri dishes.

Stems with black lesions typical of take-all were cut into 2- to 4-mm pieces. To reduce the variability of recovery from one tissue piece to another, each piece of stem was bisected vertically; one half was placed on R-PDA and the other on SM-GGT3. To isolate the pathogen from roots, a pair of adjacent segments of equal length (2-5 mm long) was cut from each infected root; one segment was placed on R-PDA and the other on SM-GGT3. Four to six pieces were placed in each dish. All dishes were incubated in the dark at 21-24 C and observed after 12-24 h. Thereafter, daily observations were made for 10 days. On R-PDA and SM-GGT3, the appearance of purple or brown halos, respectively, around tissue pieces and the emergence of hyphae were noted. Fungi emerging from tissue pieces on either medium were subcultured onto R-PDA and the appearance of purple halos noted after 24 h incubation. All fungi isolated were tested for pathogenicity as described below. Previously, Cook and Naiki (5) collected wheat plants from sites throughout the Pacific Northwest and demonstrated that the fungi capable of producing symptoms typical of take-all were *G. g. tritici*.

Pathogenicity assay. Fungi were tested for the ability to produce symptoms typical of take-all on wheat in a tube assay similar to that described previously (5). Plastic tubes were filled with 25 cm^3 of sterile vermiculite and two 4-mm diameter agar plugs from a culture were placed on the surface and then covered with a 5-cm^3 layer of vermiculite. Two wheat seeds (cv. Fielder or Hill 81) were sown in each tube and then were covered with a layer of vermiculite. Tap water (10 ml) was added to each of the tubes, which were supported in racks (200 cones per rack). Racks of tubes were covered with clear plastic and incubated at 20-25 C for 24 h to promote rapid germination of the wheat. They then were placed in growth chambers at 12-15 C with a 99% relative humidity and a 12:12 light/dark photoperiod. Plants were watered as needed with dilute Hoagland's solution (macroelements only) (18). After 3-4 wk, roots were washed free of vermiculite. The severity of take-all was evaluated on a scale of 0-8 as previously described (27), where 0 = no disease evident and 8 = plant dead or nearly so. Symptoms not characteristic of take-all were noted. All fungi were tested in at least two separate experiments. Each treatment was replicated four times, and each replication consisted of 5 or 10 cones.

Elucidation of the color change of rifampicin caused by *G. g. tritici*. Sodium ascorbate has been reported to influence certain chemical reactions involved in the metabolism of rifampicin in other organisms (13). To determine the effect of sodium ascorbate on the color change caused by *G. g. tritici* and *Rhizoctonia* spp., dPDA was prepared with phosphate buffers (27) to adjust the medium to pH 6. Sodium ascorbate was added at 0, 10, or 100 $\mu g\ ml^{-1}$, and rifampicin was added at 100 $\mu g\ ml^{-1}$. Dishes were inoculated with isolates of *G. g. tritici* (isolates 1817, WX, L108, or MV114), binucleate *Rhizoctonia* (25b-3), *R. oryzae* (241), and *R. solani* (210b-2 or C-1) as described above. Each treatment was replicated three times in the first trial and four times in the second trial. The color change was observed at 24 and 48 h after inoculation.

Thin-layer chromatography also was used to elucidate the nature of the color conversion caused by *G. g. tritici*. Two agar plugs (4-mm diameter) of cultures of isolate L108 or MV114 were placed in 50-ml Erlenmeyer flasks containing 20 ml of sterile deionized water amended with rifampicin (100 $\mu g\ ml^{-1}$). Cultures were incubated at 21 C in the dark on an orbital shaker (150 rpm) for 1, 3, 7, 12, 24, 48, and 96 h. Noninoculated water with and without rifampicin (100 $\mu g\ ml^{-1}$) and incubated for 96 h served as controls. After incubation, the liquid cultures were filtered through two layers of Whatman #4 filter paper to remove mycelia and the samples were prepared following methods adapted from Ratti et al (28). Ethyl acetate (25 ml) was added to each culture and shaken vigorously for 15 min. Virtually all pigment migrated

to the upper solvent phase, and the clear aqueous phase below was discarded. Anhydrous sodium sulfate was added to the solvent phase as necessary to remove residual water. The solvent was evaporated using a flash-evaporator (Buchler Instruments, Fort Lee, NJ). The residue was resuspended in 1 ml of methanol, transferred to a sterile glass vial and stored in the dark at 4 C until used. To limit potential chemical alterations of the samples during storage, incubation periods were coordinated so that all samples could be prepared sequentially and stored no longer than 1 h. Standard solutions were prepared by dissolving 2 mg of rifampicin or 25-desacetylrifampicin (provided by J. D. Berg, Clinical Biochemistry Department, Sandwell General Hospital, West Bromwich, U.K. and L. F. Zerilli, Lepetit Research Center, Gerenzano, Italy) in 1 ml of methanol. A glass tank lined with filter paper was filled 3 cm deep with 250 ml of a chloroform/methanol (9:1, v/v) solvent phase and equilibrated for at least 2 h before use. Twenty-five microliters of each standard and sample were spotted 2 cm from the bottom of a glass chromatography plate coated with silica gel (Sigma Chemical Co.). The plate was placed in the tank, supported with a metal rack, and the lower 1 cm submerged in solvent for 25 min until the solvent front had ascended to the top edge of the plate. The plates were observed immediately with an ultraviolet lamp (UV-366 nm, Blak-Ray lamp, UVP Inc., San Gabriel, CA) that enhanced visibility of the nonfluorescing spots. The experiment was repeated.

RESULTS

Virulence of fungal strains. Of 96 *G. g. tritici* isolates listed in Table 1, 88 were tested for ability to produce symptoms of take-all on wheat (cv. Fielder). Eighty-four isolates produced symptoms typical of take-all and disease ratings varied from 0.1 to 6.6 on the 0–8 scale. Four isolates were avirulent. All four *G. g. avenae* isolates produced typical symptoms. No non-*Gaeumannomyces* isolate produced symptoms typical of take-all. When the experiment was repeated the results were similar.

Fungal-mediated color change of rifampicin. All 96 isolates of *G. g. tritici* induced a color change from orange to purple on dPDA amended with rifampicin (100 $\mu g\ ml^{-1}$), usually within 24 h (Table 2). The intensity of the purple color varied slightly among isolates but was distinct in all cases. Isolates of the closely related fungi *G. g. avenae, G. g. graminis* and *G. incrustans* also caused the reaction as did isolates of *Leptosphaeria korrae*. However, of 106 additional isolates representing 31 species of fungi, many commonly isolated from wheat roots and crowns, only certain isolates of binucleate *Rhizoctonia, R. oryzae*, and *R. solani* caused a purple reaction (Table 2). Isolates of *Rhizoctonia* that caused the reaction represented 12 anastomosis groups and 32% of the total *Rhizoctonia* isolates tested.

Effect of rifampicin and tolclofos-methyl on the growth of *G. g. tritici* and *Rhizoctonia* spp. Tolclofos-methyl was introduced as a component of R-PDA to inhibit *Rhizoctonia* spp., thus reducing the potential for false positives. Isolates of *G. g. tritici* (WX, MV114, MV115, L108, 1817, and 1845), binucleate *Rhizoctonia* spp. (23s-2 and 25b-3), *R. oryzae* (241 and 541), and *R. solani* (C-1 and 210b-2) were grown on dPDA amended with rifampicin at 100, 175, or 250 $\mu g\ ml^{-1}$ and tolclofos-methyl at 10 and 100 $\mu g\ ml^{-1}$ in all possible combinations (data not shown). The combination of 100 $\mu g\ ml^{-1}$ rifampicin and 10 $\mu g\ ml^{-1}$ tolclofos-methyl was selected for R-PDA because it inhibited the growth of *Rhizoctonia* spp. by 98.1% (pooled value for all isolates), whereas the growth of the isolates of *G. g. tritici* was inhibited only 6.1% (pooled value for all isolates) (Table 3). Tolclofos-methyl did not interfere with the color change caused by *G. g. tritici*.

Growth of *G. g. tritici* and *Rhizoctonia* spp. on R-PDA and SM-GGT3. The growth of six isolates of *G. g. tritici* tested was inhibited significantly more on SM-GGT3 than on R-PDA (Table 3). Isolate L108 was completely inhibited on SM-GGT3. R-PDA was significantly more inhibitory to five of six strains of *Rhizoctonia* spp. than was SM-GGT3. Both of the *R. solani* isolates (C-1 and 210b-2) tested showed slight growth on SM-GGT3, but they were completely inhibited on R-PDA. *R. oryzae* isolates were inhibited less on SM-GGT3 than were other isolates of *Rhizoctonia* (Table 3).

All six isolates of *G. g. tritici* caused a purple color reaction on R-PDA, whereas only three isolates caused a brown color reaction on SM-GGT3 (Table 3). An equal number of *Rhizoctonia* isolates caused a purple color reaction on R-PDA and a brown color reaction on SM-GGT3. Isolates that caused a color reaction on SM-GGT3 did not necessarily cause a color reaction on R-PDA.

TABLE 2. Effect of fungi on the color of dPDA[y] with rifampicin (100 $\mu g/ml$)

Fungus	Anastomosis group	Number of isolates tested	Number of isolates causing a color change[z]
Aspergillus spp.		1	0
Bipolaris spp.		1	0
Cephalosporium gramineum		1	0
Fusarium culmorum		1	0
F. equiseti		1	0
F. oxysporum f. sp. *pisi* race 2		1	0
F. sambucum		1	0
F. solani		1	0
Gaeumannomyces cylindrosporus		1	0
G. graminis		1	1
G. graminis var. *avenae*		4	4
G. graminis var. *graminis*		3	3
G. graminis var. *tritici*		96	96
G. incrustans		1	1
Leptosphaeria korrae		3	3
Mucor spp.		1	0
Penicillium spp.		1	0
Pseudocercosporella herpotrichoides		1	0
Pyrenophora semeniperda (*Drechslera* state)		1	0
Pythium aristosporum		1	0
P. dissotocum		1	0
P. graminicola		1	0
P. heterothallicum		1	0
P. intermedium		1	0
P. irregulare		1	0
P. macrosporum		1	0
P. parocandrum		1	0
P. rostratum		1	0
P. sylvaticum		1	0
P. torulosum		1	0
P. ultimum var. *sporangiiferum*		1	0
P. ultimum var. *ultimum*		1	0
P. volutum		1	0
Binucleate *Rhizoctonia* spp.	AG-A	1	1
	AG-B	1	1
	AG-Ba	3	1
	AG-Bb	2	2
	AG-C	1	0
	AG-D	8	2
	AG-E	7	2
	AG-G	2	0
	AG-I	3	3
	AG-K	9	4
	AG-Q	1	0
R. oryzae	WAG-O	10	5
R. solani	AG-4	5	3
	AG-5	2	0
	AG-6 HG-I	1	0
	AG-6 GV	1	0
	AG-8	14	4
	AG-Ma	1	1
	AG-XI	4	0
Rhizopus spp.		1	0
Trichoderma spp.		1	0
T. koningii		3	0

[y] Dilute potato-dextrose agar.
[z] A color change of the medium around the inoculum plug from orange to purple occurred within 24 h for strains indicated.

Because *R. solani* commonly infects wheat roots and was a major source of false positives, an increased number of isolates were tested in a second experiment. The growth of 19 of 22 strains of *R. solani*, originally from roots of wheat or barley, was significantly less after 16 days on R-PDA than on SM-GGT3 (Table 4). Results from measurements taken at 4, 8, and 16 days showed the same trend, thus only those from 16 days are presented. All 22 isolates caused a brown reaction on SM-GGT3, whereas only 10 caused a purple reaction on R-PDA. On SM-GGT3, several of the *Rhizoctonia* isolates caused the brown reaction in as little

TABLE 3. Growth of *Gaeumannomyces graminis* var. *tritici* and *Rhizoctonia* spp. on R-PDA[t] and SM-GGT3[u]

Isolate		Growth inhibition (%)[v] R-PDA	Reaction[w]	SM-GGT3	Reaction[w]
G. g. tritici	WX	2.0 Fb[x]	+	50.0 Ca	−
	MV114	22.0 Db	+	55.3 Ca	+
	MV115	0 Fb	+	57.6 Ca	−
	L108	12.7 Eb	+	100.0 Aa	+
	1817	0 Fb	+	87.5 Ba	−
	1845	0 Fb	+	24.2 Ea	+
Binucleate *Rhizoctonia* spp.	23s-2	100.0 Aa	−	90.0 ABb	+
	25b-3	97.7 ABa	−	95.0 ABa	−
R. oryzae	241	94.3 Ca	+	51.7 Cb	+
	541	96.5 BCa	+	37.5 Db	+
R. solani	C-1	100.0 Aa	+	92.0 ABb	+
	210b-2	100.0 Aa	+	95.0 ABb	−
G. g. tritici (pooled)[y]		6.1 Bb[z]		62.4 Ba[z]	
Rhizoctonia spp. (pooled)[y]		98.1 Aa		76.3 Ab	

[t] Rifampicin (100 μg ml^{-1}) (Sigma Chemical Co.) and tolclofos-methyl (10 μg ml^{-1}) (Rizolex 75 WDG, Sumitomo Co., Japan) added to autoclaved dilute potato-dextrose agar.
[u] A semiselective medium for *G. g. tritici* reported by Juhnke et al (20).
[v] The radius of the colony was measured 11 days after inoculation. Growth inhibition on R-PDA or SM-GGT3 was quantified as the colony radius on these media expressed as a percentage of the colony radius of the same isolate on nonamended dilute potato-dextrose agar.
[w] (+) indicates a color change in the medium from orange to purple (R-PDA) within 24 h or from clear to brown (SM-GGT3) within 11 days. (−) indicates no color change after 11 days.
[x] Means in the same row followed by the same lowercase letter and means in the same column (excluding the last two means) followed by the same uppercase letter are not significantly different at $P = 0.05$, according to Fisher's protected LSD test.
[y] Means for pooled *G. g. tritici* and pooled *Rhizoctonia* spp. isolates.
[z] Pooled means in the same row followed by the same lowercase letter and pooled means in the same column followed by the same uppercase letter are not significantly different at $P = 0.05$, according to Fisher's protected LSD test.

TABLE 4. Growth at 16 days after inoculation and color reaction of *Rhizoctonia solani* on R-PDA[v] and SM-GGT3[w]

Anastomosis group	Isolate	R-PDA Reaction[x]	R-PDA Colony radius (cm)	SM-GGT3 Reaction	SM-GGT3 Colony radius (cm)
AG-4	3-6b-2	−	0 a[y]	+	4.0 b
	6-3-2	+	1.0 a	+	4.0 b
	6-3-5	+	0.6 a	+	4.0 b
	6-3-7	+	0.3 a	+	4.0 b
	6-3b-1	−	0 a	+	2.9 b
AG-5	D-1	+	1.2 a	+	2.2 b
	D-23	−	4.0 a	+	4.0 a
AG-6 HG-I	UBU-1-4	−	0.2 a	+	1.0 b
AG-6 GV	SO-2-1	−	0 a	+	1.8 b
AG-8	FAC-1727-b	−	0 a	+	3.5 b
	S1-BS1	+	0 a	+	2.6 b
	3-1b-1	+	0 a	+	4.0 b
	3-7-2	+	1.7 a	+	4.0 b
	4-5b-1	+	0.2 a	+	4.0 b
	5-6-1	−	0.1 a	+	4.0 b
	5-6-2	−	0.8 a	+	4.0 b
	5-6-3	−	0.5 a	+	4.0 b
	7-1-3s-1	−	0 a	+	2.0 b
	7-1-3s-2	−	0 a	+	1.2 a
	7-2-2	+	2.3 a	+	4.0 b
AG-Ma	4-5b-6	+	0.5 a	+	4.0 b
AG-X1	4-2b-2	−	0.5 a	+	1.8 a
Total or mean[z]	22	10	0.6 a	22	3.2 b

[v] Rifampicin (100 μg ml^{-1}) (Sigma Chemical Co.) and tolclofos-methyl (10 μg ml^{-1}) (Rizolex 75 WDG, Sumitomo Co., Japan) added to autoclaved dilute potato-dextrose agar.
[w] A semiselective medium for *G. g. tritici* reported by Juhnke et al (20).
[x] (+) indicates a color change in the medium from orange to purple (R-PDA) within 24 h or clear to brown (SM-GGT3) within 11 days. (−) indicates no color change after 11 days of incubation.
[y] Comparisons between media are only within the same strain. Means in the same row followed by the same letter are not significantly different at $P = 0.05$, according to Fisher's protected LSD tests.
[z] Total number of isolates tested and total number causing a color change; mean colony radius for all strains pooled.

as 8 h, whereas the reaction caused by *G. g. tritici* rarely occurred in less than 2–4 days. The reaction caused by isolates of *Rhizoctonia solani* on R-PDA required more than 24 h.

Isolation of *G. g. tritici* from wheat. From the Mount Vernon sample processed without surface sterilization, putative *G. g. tritici* was isolated on R-PDA from 57.6% of the stems and 13.3% of the roots; on SM-GGT3 the pathogen was isolated from 11.9% of the stems and none of the roots. When tissue pieces were surface sterilized with 1% silver nitrate, putative *G. g. tritici* was isolated on R-PDA from 66.2% of the stems and 8.3% of the roots; the fungus was isolated on SM-GGT3 from 43.1% of the stems and 3.3% of the roots (Table 5).

From the Pendleton sample processed without surface sterilization, putative *G. g. tritici* was isolated on R-PDA from 14% of the stems and 8% of the roots; the fungus was not recovered on SM-GGT3 from either stems or roots. When tissues were surface sterilized, putative *G. g. tritici* was isolated on R-PDA from 4% of the stems and 14% of the roots; the fungus was isolated on SM-GGT3 from none of the stems and 6% of the roots (Table 5).

From the Pullman sample processed without surface sterilization, putative *G. g. tritici* was isolated on R-PDA from 52.5% of the stems and 52.3% of the roots; the pathogen was isolated on SM-GGT3 from 27.7% of the stems and 18.3% of the roots. When tissues were surface sterilized, putative *G. g. tritici* was isolated on R-PDA from none of the stems and 34% of the roots; the fungus was isolated on SM-GGT3 from none of the stems and 10% of the roots (Table 5).

Recovery of putative *G. g. tritici* was consistently higher on R-PDA compared with SM-GGT3. On R-PDA the pathogen was recovered from 21.7 and 32.4% of all root and stem pieces, respectively, whereas on SM-GGT3 recovery was 6.3 and 13.8%, respectively. On R-PDA and SM-GGT3 the pathogen was isolated from 27 and 10%, respectively, of all pieces assayed. On R-PDA, 51% of all tissue pieces yielded fungal contaminants, but no bacterial contaminants were detected. On SM-GGT3, 68% of all tissue pieces sampled yielded contaminants (27% bacterial and 41% fungal). On SM-GGT3, *G. g. tritici* rarely emerged from tissue pieces that were contaminated with bacteria (Table 5).

All putative isolates of *G. g. tritici* from R-PDA or SM-GGT3 caused the color change when subcultured on R-PDA and produced symptoms typical of take-all on wheat (cv. Hill 81) in the tube assay. The average disease rating of putative *G. g. tritici* isolates recovered on R-PDA was 4.3 on the scale of 0–8,

TABLE 5. Recovery of putative *Gaeumannomyces graminis* var. *tritici* on R-PDA[n] and SM-GGT3[o] from wheat with symptoms of take-all from three commercial fields

Field	Medium	SS[p]	TS[q]	NPA[r]	RXN[s]	GGT[t]	PAT[u]	BC[v]	FC[w]	REC (%)[x]
Mt. Vernon, WA	R-PDA	None	Root	60	17	8	8	0	35	13.3
			Stem	59	46	34	34	0	20	57.6
		$AgNO_3$	Root	60	10	5	5	0	3	8.3
			Stem	65	52	43	43	0	15	66.2
	SM-GGT3	None	Root	60	1	0	0	9	2	0
			Stem	59	3	7	7	12	5	11.9
		$AgNO_3$	Root	60	1	2	2	0	3	3.3
			Stem	65	26	28	28	6	13	43.1
Pendleton, OR	R-PDA	None	Root	50	13	4	4	0	49	8.0
			Stem	50	19	7	7	0	47	14.0
		$AgNO_3$	Root	50	11	7	7	0	14	14.0
			Stem	50	19	2	2	0	49	4.0
	SM-GGT3	None	Root	50	13	0	0	0	50	0
			Stem	50	5	0	0	3	50	0
		$AgNO_3$	Root	50	18	3	3	4	36	6.0
			Stem	50	20	0	0	7	50	0
Pullman, WA	R-PDA	None	Root	109	57	57	57	0	71	52.3
			Stem	101	57	53	53	0	35	52.5
		$AgNO_3$	Root	50	30	17	17	0	7	34.0
			Stem	50	10	0	0	0	43	0
	SM-GGT3	None	Root	109	48	20	20	80	16	18.3
			Stem	101	67	28	28	56	4	27.7
		$AgNO_3$	Root	50	5	5	5	4	39	10.0
			Stem	50	7	0	0	23	39	0
Average[y]	R-PDA	None	...	...	...	...	...	...	...	33.0
		$AgNO_3$	...	...	...	...	...	...	...	21.1
			Root	...	...	...	...	...	...	21.7
			Stem	...	...	...	...	...	...	32.4
				...	...	...	...	...	...	27.0[z]
	SM-GGT3	None	...	...	...	...	...	...	...	9.7
		$AgNO_3$	...	...	...	...	...	...	...	10.4
			Root	...	...	...	...	...	...	6.3
			Stem	...	...	...	...	...	...	13.8
				...	...	...	...	...	...	10.0[z]

[n] Rifampicin (100 $\mu g\ ml^{-1}$) (Sigma Chemical Co.) and tolclofos-methyl (10 $\mu g\ ml^{-1}$) (Rizolex 75 WDG, Sumitomo Co., Japan) added to autoclaved dilute potato-dextrose agar.
[o] A semiselective medium for *G. g. tritici* reported by Juhnke et al (20).
[p] Surface sterilization procedure used: None = rinsed in water for 60 min followed by two 30 s rinses in sterile water; $AgNO_3$ = immersion in 1% silver nitrate solution for 30 s followed by a 30 s rinse in sterile water.
[q] Tissue sampled.
[r] Number of pieces assayed.
[s] Number of pieces causing a purple reaction on R-PDA or brown reaction on SM-GGT3.
[t] Number of pieces yielding putative *G. g. tritici* isolates.
[u] Number of putative *G. g. tritici* isolates causing typical take-all symptoms on wheat.
[v] Number of pieces contaminated with bacteria.
[w] Number of pieces contaminated with fungi.
[x] Percentage of total pieces from which putative *G. g. tritici* was recovered.
[y] Mean recovery from all three samples.
[z] Mean for all stem and root pieces.

with 6% of these isolates having a rating of <1. The average disease rating of isolates recovered using SM-GGT3 was 4.1 with 3% of these isolates having a rating <1.

Chromatographic analysis of rifampicin conversion by *G. g. tritici* and the effect of sodium ascorbate on the color change. Sodium ascorbate has been reported to interfere with the conversion of rifampicin to rifampicin quinone (13). Sodium ascorbate at 10 $\mu g\ ml^{-1}$ delayed but did not inhibit the color change caused by *G. g. tritici* isolates WX and L108, binucleate *Rhizoctonia* spp. 25b-3, and *R. oryzae* 241; however, 100 $\mu g\ ml^{-1}$ inhibited the reaction. The color change caused by *G. g. tritici* 1817 was delayed but not inhibited at 100 $\mu g\ ml^{-1}$ sodium ascorbate. Sodium ascorbate had no effect on *G. g. tritici* MV114, *R. solani* C-1, and *R. solani* 210b-2.

TLC was used to further elucidate the nature of the color reaction. Rifampicin migrated as an orange band with an R_F value of 0.42–0.43 in trial 1 and 0.35–0.36 in trial 2 (Table 6). Residual rifampicin was found in extracts of cultures of MV114 and L108 until 24 and 48 h, respectively. With increasing time from 1 to 48 h the intensity of the orange band decreased. Compounds migrating in an orange-red band and corresponding exactly with the 25-desacetylrifampicin standard were isolated from rifampicin-amended cultures of both MV114 and L108 beginning at 7 h. An unidentified purple compound(s) with R_F values of 0.53–0.54 in trial 1 and 0.51 in trial 2 was detected 1 h after inoculating the water containing rifampicin with *G. g. tritici*; however, the purple compound(s) was never detected in the noninoculated controls. Over time, a decrease in the concentration of rifampicin (inferred from a decrease in the intensity of the migration bands) was accompanied by an increase in the concentration of the purple compound(s). After 48 h, only purple compound(s) were detected in cultures of *G. g. tritici*. In the noninoculated control, only rifampicin was detected at 96 h.

DISCUSSION

A semiselective and diagnostic medium designated R-PDA was developed that aids in the isolation and identification of *G. g. tritici*. The diagnostic characteristic of the medium is the change in the color of rifampicin from orange to purple caused by the fungus, usually in less than 24 h. That all 96 isolates of *G. g. tritici* obtained from North America, Europe, and Australia gave a positive reaction on R-PDA strongly suggests that this trait is widespread and stable in the pathogen population. It was not surprising that the closely related fungi *G. g. avenae*, *G. g. graminis*, and *G. incrustans* also caused the reaction. The reaction appears to be uncommon among other fungi; of 31 other species tested (many of them from wheat), only *L. korrae* and *Rhizoctonia* spp. caused the color change. Thirty-two percent of the *Rhizoctonia* isolates tested caused the reaction and this trait apparently was not related to a particular species or anastomosis group. We feel that this medium will be particularly useful in studies of the epidemiology of take-all in which large numbers of fungi must be screened and *G. g. tritici* distinguished from the myriad of other fungi that inhabit wheat and barley plants.

Selectivity of the medium was enhanced by the addition of tolclofos-methyl (10 $\mu g\ ml^{-1}$) because the fungicide almost completely inhibited the growth of binucleate *Rhizoctonia* spp. and *R. solani*. *R. oryzae* also was inhibited significantly by the fungicide but on average, 42% less than *R. solani* or binucleate *Rhizoctonia* spp. Similar results of tests of tolclofos-methyl against *R. oryzae* have been reported by Kataria and Verma (22), Kataria et al (21), and Smiley et al (33,34). Because *G. g. tritici* is relatively insensitive to tolclofos-methyl, distinguishing the take-all pathogen from *Rhizoctonia* isolates on the R-PDA is very easy.

SM-GGT3 described by Juhnke et al (20) is another semiselective medium reported for *G. g. tritici*. On this medium, *G. g. tritici* and related fungi cause a brown reaction due to the utilization of L-DOPA and subsequent deposition of melanin pigments. Modifications of this medium, SM-GGT4 (10) and SM-GGT7 (11), have been reported. We chose to compare R-PDA with SM-GGT3 because apparently the modified media are no more effective than the original medium and they were described only in an abstract (10) and a short note (11).

G. g. tritici isolates grew faster and caused the characteristic color change more consistently on R-PDA than on SM-GGT3. This may be due in part to the sensitivity of *G. g. tritici* to dicloran, a component of SM-GGT3 (8). Further, SM-GGT3 was significantly less inhibitory of *Rhizoctonia* spp. than R-PDA and *Rhizoctonia* caused a color reaction more frequently on SM-GGT3 than on R-PDA. Differences in the size and diversity of *Rhizoctonia* collections screened may explain the contradictory results obtained in the current study and the study of Juhnke et al (20). Juhnke et al (20) tested an unspecified number of *Rhizoctonia* spp. and *R. solani* isolates and reported that some isolates of *R. solani* were able to cause the reaction but they were completely inhibited on SM-GGT3.

All fungi isolated from tissue pieces and designated as putative *G. g. tritici* on the basis of their growth on R-PDA caused typical symptoms of take-all on wheat. Although we did not confirm by ascospore analysis that these isolates were *G. g. tritici*, previous studies of take-all on wheat in the Pacific Northwest (5) strongly suggest it. R-PDA was consistently better than SM-GGT3 for the isolation of putative *G. g. tritici* isolates from infected tissues. For example, the average percent recovery of the pathogen from all samples on R-PDA and SM-GGT3 was 27 and 10%, respectively. Further, putative *G. g. tritici* isolates emerged more rapidly from root and stem pieces on R-PDA than from root and stem pieces on SM-GGT3. The best recovery on R-PDA occurs from samples that are processed immediately after collection, as was the case with the sample from Pullman. It is notable that R-PDA facilitated recovery of the pathogen from stubble at the Pendleton site even after the wheat had been harvested, the stubble burned, and the soil cultivated. These practices favor microbial decomposition of wheat debris and facilitate displacement of the pathogen from infested tissue by secondary colonists (14).

Because putative *G. g. tritici* isolates emerged rapidly from tissue on R-PDA, growth of other fungi usually did not interfere with the recovery of the pathogen. *Pythium* spp. were not common contaminants on R-PDA; however, technical grade metalaxyl at 1 $\mu g\ ml^{-1}$ can be added to the medium if needed without affecting the growth of *G. g. tritici* or the color reaction. Bacterial contami-

TABLE 6. Products resulting from rifampicin conversion by *Gaeumannomyces graminis* var. *tritici*[x]

Sample	Relative migration (R_F) Trial 1	Trial 2
Rifampicin	0.43	0.36
25-desacetylrifampicin	0.30	0.21
Noninoculated control[y]	0.42	0.35
Water	Not visible	Not visible
G. g. tritici MV114		
1 h	0.42; 0.53[z]	0.36; 0.51[z]
3 h	0.42; 0.53	0.36; 0.51
7 h	0.30; 0.42; 0.53	0.22; 0.36; 0.51
12 h	0.30; 0.42; 0.53	0.22; 0.36; 0.51
24 h	0.42; 0.54	0.22; 0.36; 0.51
48 h	0.54	0.51
96 h	0.53	0.51
G. g. tritici L108		
1 h	0.42; 0.53[z]	0.36; 0.51[z]
3 h	0.42; 0.53	0.36; 0.51
7 h	0.30; 0.42; 0.53	0.22; 0.32; 0.51
12 h	0.30; 0.42; 0.53	0.22; 0.32; 0.51
24 h	0.42; 0.54	0.51
48 h	0.42; 0.54	0.51
96 h	0.54	0.51

[x] *G. g. tritici* was inoculated in sterile water amended with rifampicin (100 $\mu g/ml$) and incubated for 1–96 h. Compounds were extracted with ethyl acetate and separated on thin-layer chromatography plates coated with silica gel using a chloroform/methanol (9:1, v/v) solvent system.
[y] Rifampicin incubated in sterile water without *G. g. tritici*.
[z] Purple compound(s).

nation was never a problem on R-PDA; however, it was a common problem on SM-GGT3. Surface sterilization with silver nitrate reduced but did not eliminate bacterial contamination. Bacteria growing around the tissue pieces frequently inhibited the emergence of the pathogen from the tissue and often caused a discoloration of SM-GGT3 that could be confused with the color change caused by *G. g. tritici*. When tissue pieces, overgrown with bacteria on SM-GGT3, were transferred to R-PDA, the pathogen often emerged from the tissue.

The occurrence of positive color reactions around pieces of tissue that subsequently did not yield putative *G. g. tritici* isolates was common on both media. Growth of the pathogen inside the pieces may have been sufficient to cause the reaction even when contaminants inhibited the pathogen from emerging from the tissue. Another possibility is that metabolites of the pathogen capable of causing the reaction persisted in the tissue after the pathogen died. On SM-GGT3, false positives occasionally yielded fungal contaminants that caused the brown reaction. Other fungi, including some ascomycetes, have melanin biosynthetic pathways similar to those of *G. g. tritici* (3,12) and may also be able to utilize L-DOPA. On R-PDA, however, false positives rarely yielded fungal contaminants that caused the reaction when subcultured on fresh plates of R-PDA. The most common contaminants on both media were *Fusarium* spp. and a nonsporulating fungus with lightly pigmented mycelia.

Our results indicate that *G. g. tritici* completely converts or degrades rifampicin. After 2–3 days of incubating rifampicin in the presence of *G. g. tritici*, the antibiotic was not detected in extracts of liquid cultures of the fungus using thin-layer chromatography. Furthermore, the gradual disappearance of rifampicin was accompanied by the accumulation of an unidentified purple compound(s). This compound may be an oxidation-product because oxidation of rifampicin by ammonium persulfate or ferric nitrate results in a color change of the antibiotic from orange to purple under alkaline conditions (13). Desacetylrifampicin, which is the principal product of rifampicin metabolism in animals (26), also was detected. Sodium ascorbate, which has been reported to interfere with the reduction of rifampicin to rifampicin quinone (13), delayed but did not always inhibit the purple reaction in vitro. This may indicate the generation of small amounts of rifampicin quinone; however, samples of this compound were not available for testing. Further analysis will be required to determine the principal mode of rifampicin degradation by *G. g. tritici*.

LITERATURE CITED

1. Asher, M. J. C. 1980. Variation in pathogenicity and cultural characteristics in *Gaeumannomyces graminis* var. *tritici*. Trans. Br. Mycol. Soc. 75:213-220.
2. Asher, M. J. C., and Shipton, P. J, eds. 1981. Biology and Control of Take-all. Academic Press, New York.
3. Bell, A. A., and Wheeler, M. H. 1986. Biosynthesis and functions of fungal melanins. Annu. Rev. Phytopathol. 24:411-451.
4. Chamswarng, C., and Cook, R. J. 1985. Identification and comparative pathogenicity of *Pythium* species from wheat roots and wheat-field soils in the Pacific Northwest. Phytopathology 75:821-827.
5. Cook, R. J., and Naiki, T. 1982. Virulence of *Gaeumannomyces graminis* var. *tritici* from fields under short-term and long-term wheat cultivation in the Pacific Northwest, U.S.A. Plant Pathol. 31:201-207.
6. Cook, R. J., and Veseth, R. J. 1991. Wheat Health Management. American Phytopathological Society, St. Paul, MN.
7. Cunningham, P. C. 1981. Isolation and culture. Pages 103-124 in: Biology and Control of Take-all. M. J. C. Asher and P. J. Shipton, eds. Academic Press, New York.
8. Duffy, B. K. 1992. A new selective medium for *Gaeumannomyces graminis* var. *tritici*, and evaluation of combinations of *Pseudomonas* spp. and fungal biocontrol agents for take-all suppression. M. S. thesis. Washington State University, Pullman, WA.
9. Duffy, B. K., and Weller, D. M. 1991. A semi-selective medium for identification of *Gaeumannomyces graminis* var. *tritici*. (Abstr.) Phytopathology 81:1168.
10. Elliott, M. L. 1989. An improved selective medium for isolation of *Gaeumannomyces*-like fungi. (Abstr.) Phytopathology 79:1177.
11. Elliott, M. L. 1991. A selective medium for *Gaeumannomyces*-fungi. Plant Dis. 75:1075.
12. Elliott, M. L. 1991. Effect of melanin biosynthesis inhibitors on *Gaeumannomyces* spp. and *Magnaporthe poae*. Phytopathology 81:1168.
13. Gallo, G. G., and Radaelli, P. 1976. Rifampin. Pages 467-513 in: Analytical Profiles of Drug Substances, Vol. 5. K. Florey, ed. Academic Press, New York.
14. Garrett, S. D. 1970. Pathogenic Root-Infecting Fungi. Cambridge University Press, Cambridge.
15. Henson, J. M. 1989. DNA probe for identification of the take-all fungus, *Gaeumannomyces graminis*. Appl. Environ. Microbiol. 55:284-288.
16. Henson, J. M. 1992. DNA hybridization and PCR testing of *Gaeumannomyces, Phialophora,* and *Magnaporthe* isolates. Mycol. Res. 96:629-636.
17. Henson, J. M., Goins, T., Grey, W., Mathre, D. E., and Elliott, M. L. 1993. Use of polymerase chain reaction to detect *Gaeumannomyces graminis* DNA in plants grown in artificially and naturally infested soil. Phytopathology 83:283-287.
18. Hoagland, D. R., and Arnon, D. I. 1950. The water-culture method for growing plants without soil. Cal. Agr. Exp. Sta. Circ. 347.
19. Ingram, D. M., and Cook, R. J. 1990. Pathogenicity of four *Pythium* species to wheat, barley, peas and lentils. Plant Pathol. 39:110-117.
20. Juhnke, M. E., Mathre, D. E., and Sands, D. C. 1984. A selective medium for *Gaeumannomyces graminis* var. *tritici*. Plant Dis. 68:233-236.
21. Kataria, H. R., Hugelshofer, U., and Gisi, U. 1991. Sensitivity of *Rhizoctonia* species to different fungicides. Plant Pathol. 40:203-211.
22. Kataria, H. R., and Verma, P. R. 1990. Efficacy of fungal seed treatments against pre-emergence damping-off and post-emergence seedling root rot of growth chamber grown canola caused by *Rhizoctonia solani* AG-2-1 and AG-4. Can. J. Plant Pathol. 12:409-416.
23. Moore, K. J., and Cook, R. J. 1984. Increased take-all of wheat with direct drilling in the Pacific Northwest. Phytopathology 74:1044-1049.
24. O'Dell, M., Flavell, R. B., and Hollins, T. W. 1992. The classification of isolates of *Gaeumannomyces graminis* from wheat, rye and oats using restriction fragment length polymorphisms in families of repeated DNA sequences. Plant Pathol. 41:554-562.
25. Ogoshi, A., Cook, R. J., and Bassett, E. N. 1990. *Rhizoctonia* species and anastomosis groups causing root rot of wheat and barley in the Pacific Northwest. Phytopathology 80:784-788.
26. Oldfieled, S., Berg, J. D., Stiles, H. J., and Buckley, B. M. 1986. Measurement of rifampicin and 25-desacetylrifampicin in biological fluids using high-performance liquid chromatography with direct sample injection. J. Chromatogr. 377:423-429.
27. Ownley, B. H., Weller, D. M., and Thomashow, L. S. 1992. Influence of in situ and in vitro pH on suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* 2-79. Phytopathology 82:178-184.
28. Ratti, B., Parenti, R. R., Toselli, A., and Zerilli, L. F. 1981. Quantitative assay of rifampicin and its main metabolite 25-desacetylrifampicin in human plasma by reversed-phase high performance liquid chromatography. J. Chromatogr. 225:526-531.
29. SAS Institute, Inc. 1985. SAS User's Guide: Basics. SAS Institute, Inc., Cary, NC.
30. SAS Institute, Inc. 1985. SAS User's Guide: Statistics. SAS Institute, Inc., Cary, NC.
31. SAS Institute, Inc. 1986. SAS System for Linear Models. SAS Institute, Inc., Cary, NC.
32. Schesser, K., Luder, A., and Henson, J. M. 1991. Use of polymerase chain reaction to detect the take-all fungus, *Gaeumannomyces graminis,* in infected wheat plants. Appl. Environ. Microbiol. 57:553-556.
33. Smiley, R. W., Uddin, W., Ott, S., and Rhinhart, K. E. L. 1990. Influence of flutolanil and tolclofos-methyl on root and culm diseases of winter wheat. Plant Dis. 74:788-791.
34. Smiley, R. W., Wilkins, D. E., and Klepper, E. L. 1990. Impact of fungicide seed treatments on Rhizoctonia root rot, take-all, and growth of winter wheat. Plant Dis. 74:782-787.