Ecology and Epidemiology

Characterization of Isolates of *Rhizoctonia solani* from Cereal Roots in South Australia and New South Wales

A. D. Rovira, A. Ogoshi, and H. J. McDonald

First and third authors, CSIRO Division of Soils, Glen Osmond, South Australia 5064; second author, Faculty of Agriculture, Hokkaido University, Kita 9, Nishi 9, Kita-Ku, Sapporo 060, Japan.

We thank R. J. & O. Manley from whose farm many isolates were obtained; R. A. Fischer, CSIRO Division of Plant Industry, Canberra; and J. Dickinson, S. A. Department of Agriculture, Port Lincoln, who submitted plant samples.

Accepted for publication 22 January 1986.

ABSTRACT

Rovira, A. D., Ogoshi, A., and McDonald, H. J. 1986. Characterization of isolates of *Rhizoctonia solani* from cereal roots in South Australia and New South Wales. Phytopathology 76:1245-1248.

Highly pathogenic isolates of *Rhizoctonia solani* from roots of wheat and barley from widely separated areas of South Australia and one site in New South Wales have been shown to belong to a single anastomosis group. These isolates readily anastomosed with AG-BI and rarely with AG-2-1 and AG-2-2 IIIB, yet differed from these anastomosis groups in cultural characteristics and are thiamine autotrophic. These results support an earlier proposal that a new anastomosis group, AG-8, be formed to accommodate these isolates of *R. solani*, which cause severe losses to cereal crops in southern Australia.

Additional key words: *Thanatephorus cucumeris*.

Rhizoctonia bare patch caused by *Rhizoctonia solani* Kühn is more severe in cereals and other crops sown by direct drilling (no till) than where sown after conventional cultivation (2,10–13, 16). With the growing acceptance of conservation tillage, which includes direct drilling or no-till, by farmers in many parts of the world, it is likely that crop losses from root rot caused by *R. solani* will increase. This will lead to a search for control by chemicals and resistant varieties and control by such means could be facilitated by having a better understanding of the anastomosis groups within *R. solani*. Neate (4) reported that the two major Rhizoctonias from wheat fields in South Australia were the multinucleate *R. solani* (*Thanatephorus cucumeris* (Frank) Donk) and binucleate Rhizoctonia-like fungi identified as *Ceratobasidium cornigerum* (Bourd.) Rogers, but there were large differences in the proportions of these two species in two wheat fields separated by some 300 km on different soil types having different rainfall patterns and crop rotations. Isolates of *R. solani* were pathogenic on six plant species, whereas pathogenicity of isolates of *C. cornigerum* depended on the plant species and were not pathogenic to wheat, even when isolated from wheat roots (4). Neate and Warcup (5) found that most isolates of *T. cucumeris* from cereals in South Australia did not anastomose with any of the groups AG-1 to AG-7 but anastomosed readily with each other; they proposed a new group, AG-8, to accommodate these.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

©1986 The American Phytopathological Society

The collaborative study reported in this paper began in 1983, two years after it had been found that none of the 25 isolates of *R. solani* from plants in patches of poor growth in direct drilled wheat (*Triticum aestivum* L.) anastomosed with the groups AG-1, AG-2-1, AG-2-2, AG-3, AG-4, and AG-5 (10,11). This study used Japanese isolates of *R. solani* belonging to recognized anastomosis groups to determine if isolates from diseased roots of wheat and barley from widely separated fields in South Australia and one isolate from direct drilled wheat in New South Wales belong to these anastomosis groups and to compare cultural characteristics and thiamine requirements of the isolates.

MATERIALS AND METHODS

Isolates. The isolates used in this study were obtained from roots of wheat and barley (*Hordeum vulgare* L.) showing severe root rot and truncation (10); they were identified as *R. solani* on cultural and morphological characteristics (9). Isolates were obtained from wheat plants collected over an area of 4 ha from the CSIRO Avon Experimental Site, South Australia (34° 14′S, 138° 18′E). Other isolations were made from roots of wheat at Streaky Bay, South Australia (32° 45′S, 134° 15′E), and from roots of barley at Avon and at Kielpa, South Australia (33° 36′S, 136° 15′E), where severe disease occurred. The isolate from Yanco, New South Wales (34° 36′S, 146° 25′E), came from plants with poor growth in direct drilled wheat in experimental plots in tillage trials conducted by R. A. Fischer, CSIRO.

In the exploratory study, six isolates of *R. solani* from direct drilled wheat in a long-term rotation and tillage trial were investigated. In the second, more detailed study, five isolates (isolates 21, 68, 109, 121, and 125) from wheat and barley roots from four widely separated sites were used. The Australian isolates of *R. solani*, together with information on host, geographic origin, and pathogenicity are listed in Table 1.

Staining of nuclei. The number of nuclei in hyphae were determined by taking hyphae from the edge of a colony from one-sixth strength neutral Dox yeast agar (NDY) (15) (0.33 g $NaNO_3$, 0.16 g NH_2PO_4, 0.08 g $MgSO_4$, 0.08 g KCl, 0.08 g yeast extract, 5.0 g sucrose, 15.0 g agar, (0.1%) 1.7 ml $FeSO_4$, and 1 L water), staining with 1% aniline blue mixed 50% glycerol slightly acidified with HCl, and heating gently (14).

Pathogenicity testing. The pathogenicity and virulence of isolates of *R. solani* were tested against wheat, peas (*Pisum* sativum L.), medic (*Medicago truncatula* Gaertn.), and ryegrass (*Lolium rigidum* Gaud.) with colonized agar plugs as inoculum and heat sterilized calcareous sandy loam in the bioassay apparatus previously described (10). A root damage rating scale of 0 to 5 (3) was used to assess the pathogenicity of the Australian isolates toward the four hosts. Values of 3, 4, and 5 represent a 30, 50, and >80% reduction in root length, respectively, compared with that of the control in sterilized soil with no disease (rating = 0).

TABLE 1. Pathogenicity of Australian isolates of *Rhizoctonia solani* from wheat and barley toward wheat, peas, medic, and ryegrass

Isolate	Host	Geographic origin	Perfect stage[b]	Disease rating[a] Wheat	Peas	Medic	Ryegrass
From one field							
C	Wheat	Avon, S. A.	−	2.0	0.4	1.0	1.0
11	Wheat	Avon, S. A.	+	4.2	4.0	3.0	3.0
21	Wheat	Avon, S. A.	+	5.0	5.0	1.5	3.0
27	Wheat	Avon, S. A.	+	5.0	4.8	5.0	4.0
30	Wheat	Avon, S. A.	−	4.2	4.0	2.8	4.0
D	Wheat	Avon, S. A.	+	2.0	0.4	5.0	5.0
From different fields							
21	Wheat	Avon, S. A.	+	5.0	5.0	1.5	3.0
68	Wheat	Streaky Bay, S. A.	−	3.8	4.5	3.0	1.9
109	Barley	Kielpa, S. A.	+	3.2	1.6	1.0	2.5
121	Barley	Avon, S. A.	−	3.2	1.5	0.5	2.8
125	Wheat	Yanco, N. S. W.	+	3.3	3.2	3.8	3.0

[a] On a 0 to 5 scale (3).
[b] *Thanatephorus cucumeris.*

For isolations, selected roots showing damage were cut into segments, which were washed in sterile distilled water, plated onto one-sixth strength NDY agar, and incubated at 25 C.

Anastomosis grouping. The Australian isolates were tested for their ability to anastomose with each other and with 21 Japanese isolates belonging to eight AG groups, viz., AG-1 to AG-7 and AG-BI.

Anastomosis was tested by taking 5-mm-diameter disks from the growing margins of young colonies on potato-dextrose agar (PDA) and placing them 3 cm apart in 9-cm-diameter petri dishes with tap water agar. The plates were incubated at 20 C until advancing hyphae made contact and slightly overlapped, at which stage these overlapping hyphae were observed under the microscope for anastomosis (6,8).

Cultural characteristics. A 5-mm agar plug from the growing edge of the culture growing on PDA was transferred to the edge of a 10-cm-diameter petri dish of PDA, which was incubated for 2 wk at 25 C in the dark.

Thiamine requirement. A 5-mm agar plug from the growing edge of a culture on PDA was transferred to the edge of a 9-cm-diameter petri dish of Czapek–Dok agar with and without 10^{-5} M thiamine hydrochloride; growth was observed after 2 wk at 25 C (7).

RESULTS

Pathogenicity of isolates. All Australian isolates were pathogenic on wheat, peas, medic, and ryegrass, with control plants rating 0 and inoculated plants rating from 0.4 to 5 (Table 1). Isolations from damaged roots produced *R. solani*.

Anastomosis grouping. The six isolates from roots of wheat from Avon anastomosed freely with each other but did not anastomose with members of AG-1 IA (CS-2), AG-1 IB (B-39), AG-1 IC (FE-3), AG-3 (ST-9), AG-4 (ARS-5), AG-5 (GM-10), and AG-7 (1535); isolate C fused occasionally with AG-2-1. These preliminary results indicated that these Australian isolates belonged to a single anastomosis group with doubtful relationship to recognized groups.

TABLE 2. Anastomosis between Australian and Japanese isolates of *Rhizoctonia solani*

Group	Japanese isolate	Australian isolate[z] 21	68	109	121	125
AG-2-1	FC-2	a	a	a	a	a
	SH-20	a	b	c	a	a
	TG-1	b	a	a	a	a
	HV-1	a	a	a	a	a
AG-2-2 IIIB	C-55	b	a	a	a	c
	C-96	a	c	c	b	b
	C-100	a	a	a	a	a
	C116	a	c	b	b	a
AG-2-2 IV	R-126	a	a	a	a	a
	RI-64	a	a	a	a	a
	B-62	a	a	a	a	a
	B-70	a	a	a	a	a
AG-6	OT-2-1	a	b	a	a	a
	Ao-1-6	a	a	a	a	a
	T-31	a	a	a	a	a
	YK-3-3	a	a	a	a	a
AG-BI	AK-1-4	c	b	c	a	c
	CA-2-1	a	c	c	a	c
	No. 70	c	b	c	a	c
	TS-2-4	c	c	c	b	c
	SN-1-2	a	b	b	a	c

[z] a: No fusion. b: Killing reaction where the contacting cells died while the contacted cell was alive. It is doubtful if this is anastomosis. c: Killing reaction where both fused cells died—this is defined as anastomosis. Where this occurred the frequency was about 1% of that found with isolates of the same anastomosis group.

This led to the more detailed study with isolates from wheat and barley from several geographically different sites against a selection of Japanese cultures representing AG-2-1, AG-2-2, AG-6, and AG-BI isolates. These groups were selected because Kuninaga et al (1) had shown that AG-BI anastomosed with members of AG-2-1, AG-2-2, and AG-6. The results (Table 2) show that there is anastomosis between Australian isolates and the AG-BI isolates. However, the frequency of anastomosis is extremely low, about 1% of that observed between isolates of the same anastomosis group.

TABLE 3. Growth of isolates of *Rhizoctonia solani* on thiamine-free Czapek–Dox agar

Isolate	Growth and pigmentation
Japanese	
AG-2-2 IIIB	–
AG-2-2 IV	–
AG-5	–
AG-BI	–
AG-2-1	+
Australian	
C	+
D	+
11	+
21	+
27	+
30	+
69	+
109	+
121	+
125	+

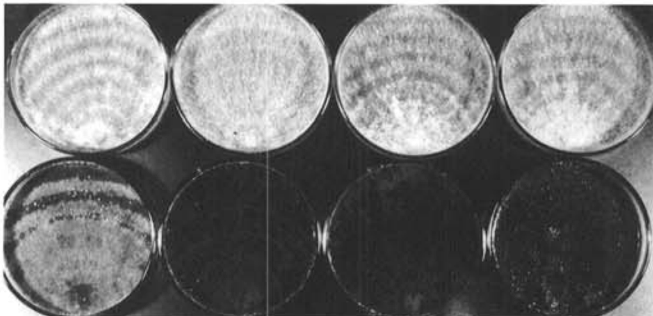

Fig. 1. Cultural characteristics of *Rhizoctonia solani* isolates on potato dextrose agar for 2 wk. Top row from left to right: isolates 21, 68, 109, and 125, respectively. Bottom row, AG-2-1, AG-2-2 IIIB, AG-2-2 IV, and AG-BI, respectively.

Cultural characteristics. The Australian isolates were identical with each other on PDA; the colonies were whitish at first and turned to light brown after 2 wk and showed concentric zonation (Fig. 1). Sclerotia were not distinctive. These features somewhat resemble those of AG-5, but isolates of AG-5 did not anastomose with the Australian isolates. On PDA, the Japanese isolates belonging to AG-2-2 IIIB, AG-2-2 IV, and AG-BI were dark brown and the AG-2-1 isolates were a medium brown (Fig. 1). On Czapek–Dox agar the colonies of the Australian isolates became brownish with abundant aerial mycelia. Growth on agar media by the Australian isolates was slower than that of isolates from other anastomosis groups. All the Australian isolates were multinucleate.

Thiamine requirement. All the Australian and Japanese isolates belonging to AG-2-1 were thiamine autotrophic, whereas the Japanese isolates of AG-2-2 IIIB and AG-BI were auxotrophic (Table 3). Thiamine requirement is a feature of the anastomosis group rather than of the isolate and hence has been considered a good feature for distinguishing between groups (7). Figure 2 shows the growth of three Japanese and three Australian isolates on Czapek-Dox agar with and without thiamine.

DISCUSSION

We conclude that the Australian isolates of *R. solani* from cereal roots that we tested belong to an anastomosis group different from known anastomosis groups and, in this way, our findings support the proposal (5) that a new anastomosis group, AG-8, be formed. The very low frequency of anastomosis between the Australian isolates and AG-BI isolates together with the occasional anastomosis with AG-2-1 and AG-2-2 IIIB, means that the Australian cultures share some of the characteristics of bridging isolates (AG-BI), but they differ in requiring thiamine for growth. However, as the Australian isolates form the major group associated with severe root disease over a large part of southern Australia, they should be considered to belong to group AG-8 proposed by Neate and Warcup (5), and belong to a group bridging with Japanese isolates of relatively low pathogenicity.

LITERATURE CITED

1. Kuninaga, S., Yokosawa, R., and Ogoshi, A. 1979. Some properties of anastomosis Group 6 and BI in *Rhizoctonia solani* Kühn. Ann. Phytopathol. Soc. Jpn. 45:207-214.
2. MacNish, G. C. 1985. Methods of reducing rhizoctonia patch of cereals in Western Australia. Plant Pathol. 34:355-359.
3. McDonald, H. J., and Rovira, A. D. 1985. Development of inoculation technique for *Rhizoctonia solani* and its application to screening cereal cultivars for resistance. Pages 174-176 in: Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Rovira, K. J. Moore,

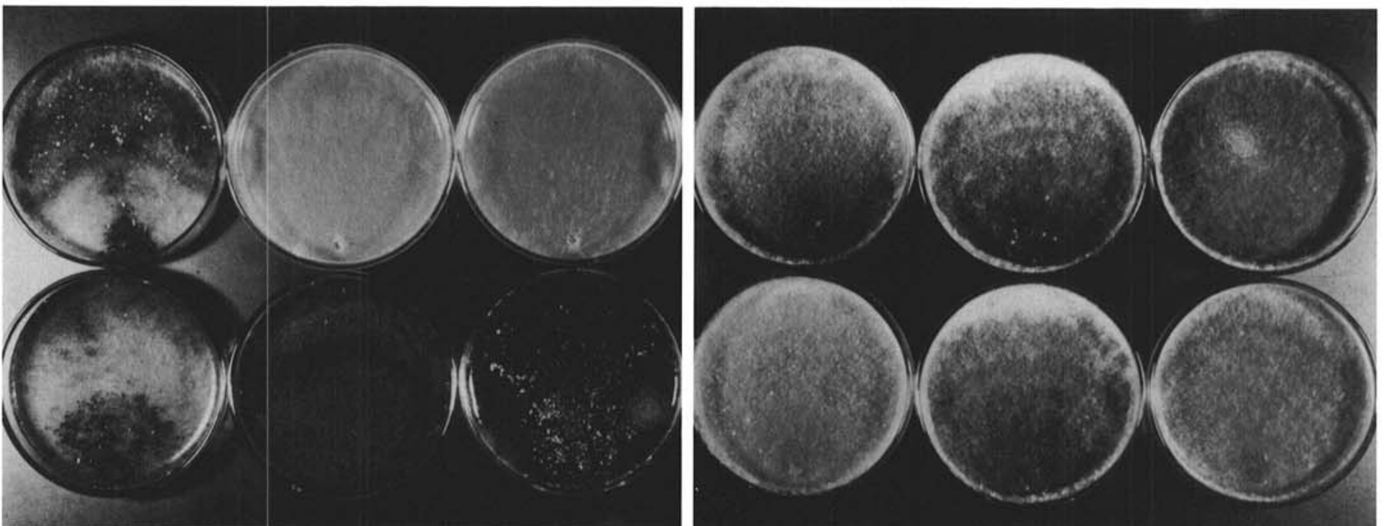

Fig. 2. Growth of *Rhizoctonia solani* on Czapek-Dox medium with and without thiamine. Top row, medium without thiamine. Bottom row, medium with thiamine. Cultures of *R. solani* are (left to right) Japanese isolates AG-2-1, AG-2-2 IIIB, AG-BI; and Australian isolates 21, 109, and 125, respectively.

P. T. W. Wong, and J. F. Kollmorgen, eds. Amer. Phytopathol. Soc., St. Paul, 358 pp.
4. Neate, S. M. 1985. Rhizoctonia in South Australian wheat fields. Pages 54-56 in: Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Rovira, K. J. Moore, P. T. W. Wong, and J. F. Kollmorgen, eds. Amer. Phytopathol. Soc., St. Paul. 358 pp.
5. Neate, S. M., and Warcup, J. H. 1985. Anastomosis grouping of some isolates of *Thanatephorus cucumeris* from agricultural soils in South Australia. Trans. Br. Mycol. Soc.
6. Ogoshi, A. 1976. Studies on the grouping of *Rhizoctonia solani* Kühn with hyphal anastomosis and on the perfect stages of groups. Bull. Nat. Inst. Agric. Sci. Tokyo, Series C30. 65 pp.
7. Ogoshi, A., and Ui, T. 1978. Specificity in vitamin requirement among anastomosis groups of *Rhizoctonia solani* Kühn. Ann. Phytopathol. Soc. Jpn. 45:47-53.
8. Ogoshi, A., Oniki, M., Sakai, R., and Ui, T. 1979. Anastomisis grouping among isolates of binucleate *Rhizoctonia*. Trans. Mycol. Soc. Jpn. 20:33-39.
9. Parmeter, J. R., Jr., and Whitney, H. S. 1970. Taxonomy and nomenclature of the imperfect state. Pages 7-19 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, Jr., ed. University of California Press, Berkeley.
10. Rovira, A. D. 1986. Influence of crop rotation and tillage on Rhizoctonia bare patch of wheat. Phytopathology 76:669-673.
11. Rovira, A. D., and Venn, N. R. 1985. Effect of rotation and tillage on take-all and Rhizoctonia root rot in wheat. Pages 255-258 in: Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Rovira, K. J. Moore, P. T. W. Wong, and J. F. Kollmorgen, eds. Amer. Phytopathol. Soc., St. Paul. 358 pp.
12. Sumner, D. R. 1977. Influence of tillage practices on root diseases of southern pea (*Vigna unguiculata*). Annu. Rep. Bean Improv. Coop. 20:91-93.
13. Sumner, D. R., Doupnik, B., and Boosalis, M. G. 1981. Effects of reduced tillage and multiple cropping on plant diseases. Annu. Rev. Phytopathol. 19:167-187.
14. Tu, C. C., and Kimbrough, J. W. 1973. A rapid staining method for *Rhizoctonia solani* and related fungi. Mycologia 65:941-944.
15. Warcup, J. H. 1955. Isolation of fungi from hyphae present in soil. Nature (London) 175:953.
16. Weller, D. M., Cook, R. J., MacNish, G., Bassett, E. N., Powelson, R. L., and Peterson, R. R. 1986. Rhizoctonia root rot of small grains favored by reduced tillage in the Pacific Northwest. Plant Dis. 70:70-73.