Identity and pathogenicity of Rhizoctonia spp. associated with bare patch disease of cereals at a field site in Western Australia

F.A. ROBERTS and K. SIVASITHAMPARAM

Soil Science and Plant Nutrition Group, School of Agriculture, University of Western Australia, Nedlands, W.A. 6009, Australia

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Abstract

Roots of seedlings of wheat and barley affected by bare patch disease at a field site in Western Australia were assessed for root damage and plated to isolate fungi. The patches were variable in shape and size and had the most severely affected plants in the centre. Of the 165 isolates of *Rhizoctonia* spp. obtained, 90% were multinucleate and 10% binucleate, the former being predominant in the plants at the centre of the patch. The relative frequency of binucleate isolates increased with proximity to the periphery. The increase in activity of avirulent binucleate isolates towards the periphery of the patch may be related to the sharp and abrupt edging of the patch. A variety of other species of fungi such as *Fusarium* spp., *Mortierella* spp., *Bipolaris sorokiniana*, *Pythium* sp. and *Trichoderma* sp. were encountered within the patches. The multinucleate isolates belonging to anastomosis groups (Ag) 2-1, 2-2 and 8 (*Thanatephorus cucumeris*) were most pathogenic to wheat. The binucleate isolates of Ag C, D, E, and K (*Ceratobasidium* sp.) were less pathogenic. It is suggested that the bare patch disease is caused by a complex of root rot fungi composed of one or more anastomosis groups of *Rhizoctonia* spp. and other associated fungi.

Additional keywords: Thanatephorus cucumeris, Ceratobasidium sp.

Introduction

Rhizoctonia solani Kühn (teleomorph: Thanatephorus cucumeris Frank (Donk)) has been recorded as the causal organism of the 'bare patch' disease of cereals in South Australia (Samuel, 1928; Samuel and Garrett, 1932; Kerr, 1955; Flentje, 1956; De Beer, 1965), in Canada (Benedict and Mountain, 1956), in USA (Weller et al., 1986) and in England (Dillon-Weston and Garrett, 1943), of 'stunted patch' in Victoria (Price, 1970), stunt disorders in Scotland (Murray and Nicholson, 1979), and 'purple patch' in New South Wales (Hynes, 1933, 1937). In South Africa, a disease similar to bare patch has been observed by Scott et al. (1979) who named it 'crater disease'. They implicated R. solani, R. cerealis van der Hoeven, Periconia macrospinosa Lefebvre & Johnson and Pythium oligandrum Drechsler as causal organisms. Banyer (1966) observed stunted plants occurring in distinct patches that tend to be circular in shape in South Australian districts. Smith et al. (1984) characterized the field symptoms on wheat in South Africa as 'patches of chlorotic stunted plants appearing about 4 weeks

after planting'. Shier et al. (1963) recorded several bare patches caused by *Rhizoctonia* spp. in their nitrogen rates experiment in Western Australia. MacNish (1983) reported a positive identification of bare patch in Western Australia in 1971 affecting wheat and barley. Since then, the disease has been observed in oats, rye, triticale, *Medicago littoralis* Rhode, lupins and rapeseed. He noted that it was severe on the calcareous mallee soils in the region of Salmon Gums.

As identification of *Rhizoctonia* spp. associated with bare patch has not been attempted before in Western Australia, this investigation is aimed to study the identity and pathogenicity of *Rhizoctonia* spp. associated with bare patch disease of wheat and barley in a farm at Lake Grace, Western Australia.

Materials and methods

Plant sampling. Observations of and sampling from wheat and barley plants in bare patches were made during July, 1984 in Lake Grace, Western Australia. For circular patches, the radius was obtained by running a measuring tape from the centre to the edge of the patch. For non-circular patches, the diameter was obtained by locating the centre of the patch and measuring the distance on two perpendicular axes equidistant from the centre to the edge. Twelve patches were randomly picked and measured.

Plants sampled were at the four-to five-leaf-stage and were 5 to 6 weeks old. The patch was divided into three concentric zones 'A', 'B' and 'C' for sampling where 'C' consisted of plants at the centre of the patch, 'A' plants at the periphery of the patch, and 'B' those in between 'A' and 'C'. Plants outside the bare patch served as control.

Roots of wheat and barley were washed free of soil under running water, floated in a white enamel dish of water and extent of root disease was scored under low magnification. Ratings were made on a 1 to 5 scale at 0.5 intervals, 0 being healthy and 5 indicating rotting of all roots. The average percentage root disease index was calculated using a modified method of McKinney (1923).

Fungal isolation and identification. The fungi were isolated using the tissue-transplant method described by Riker and Riker (1936). Small sections of the roots about 0.5 cm long were cut and surface-sterilized for 10 minutes with 0.125% sodium hypochlorite, rinsed in three changes of sterile, distilled water, plated on potato dextrose agar (PDA) and water agar (WA) both containing streptomycin sulphate. Plates were incubated at 25 °C. Colonies which appeared two days later were subcultured onto fresh PDA plates. The frequency of occurrence of all fungi isolated was recorded.

Colonies resembling *Rhizoctonia* spp. were transferred to PDA. Fungal characteristics listed by Parmeter and Whitney (1970) were used to delineate isolates to species. The number of nuclei per hyphal cell and septal morphology were determined for each isolate by using the rapid staining technique described by Tu and Kimbrough (1973) and Burpee et al. (1980). Hyphal morhology and branching patterns were observed on the same slides used for the determination of nuclear number. Mycelial growth rate was determined by transferring a 5 mm diameter mycelial plug of each isolate onto PDA and incubating at 25 °C. Three replications of each isolate were used. Colony diameters were measured after 72 hours. The same cultures were incubated and examined after four weeks for the presence of monilioid cells and colony pigmentation.

Isolates were induced to produce basidia using Murray's (1982) method where isolates were grown on dextrose marmite agar, transferred onto 2% WA and cornmeal agar (CMA) and incubated at 24 °C during the day with diffuse light and at 10 °C at night. Dimensions of basidia, basidiospores, sterigmata and width of supporting hypha below basidium were measured.

Anastomosis grouping. Anastomosis grouping for multinucleate and binucleate isolates was determined by using the method of Parmeter et al. (1969). Each multinucleate isolate was paired separately with each tester strain on glass slides coated with 2% water agar, incubated at 25 °C for 48 to 72 hours, and then stained with 0.5% aniline blue in lactophenol and examined for anastomosis. Similarly, binucleate isolates were paired separately with each binucleate tester stain. Isolates were assigned to a particular anastomosis group (Ag) only if cell death accompanied hyphal fusion. Tester strains used for multinucleate isolates were those of Professor A. Ogoshi of Hokkaido University, Japan, namely Ag 2-1 (TG 1, PS4), Ag 2-2 (C127), Ag 3 (RS30), Ag 4 (SNI), Ag 5 (R470), Ag 6 (A01-6), Ag 7 (1535) and Ag Bl (CA2-1) except for Ag 8 which was supplied by Dr S. Neate of the Commonwealth Scientific and Industrial Research Organization (CSIRO), South Australia. Binucleate tester strains used were Ag A (SH 6), Ag Bb (C348), Ag C (54D25), Ag D (C57) Ag E (F18), Ag F (SIR1), Ag G (4D9) and Ag K (AC1). These tester strains were supplied by Drs D.H. Wong and S. Neate.

Pathogenicity tests. Thirteen isolates of binucleate and multinucleate Rhizoctonia spp. were tested for their pathogenicity on wheat under glasshouse conditions. The isolates tested were multinucleate Ag 2-1, Ag 2-2, Ag 5, Ag 6, Ag Bl and Ag 8; and binucleate Ag D, Ag E, Ag K and an unassigned isolate.

Inoculum was prepared by growing the test fungi on moist sterile millet seeds (Panicum miliaceum L.) for two weeks at room temperature. The inoculum was then mixed thoroughly (0.2% w/w) with pasteurized (aerated steam at 60 °C for 30 min) sub-soil yellow sand. Control pots only contained sterile millet seed. Each plastic pot (7.5 cm diameter) was filled with 200 g of pasteurized inoculated sub-soil yellow sand, sown with 10 surface-sterilized germinated seeds of wheat cv. Gamenya and covered with a thin layer of pasteurized soil. Six replicates were used for each treatment. These pots were placed in a phytotron cabinet with day and night temperatures of 18 and 15 °C respectively. The soil was maintained at 65% water holding capacity by watering daily to a constant weight with deionized water.

Four weeks after sowing, surviving plants were counted, roots washed free of soil and rated for root infection as described in the section on plant sampling.

Results

Plant sampling. Distinct dying patches of wheat and barley plants with bare patch symptoms typical for *Rhizoctonia* were observed in the paddocks. The patches varied in length (3.1 to 19.5 m, av. 9.0 m), width (1.2 to 5.5 m, av. 2.9 m) and area (2.9 to 55.2 m², av. 23.4 m²). Diseased plants contrasted sharply in size and vigour with surrounding 'healthy' wheat and barley plants. Patches were circular to irregular in shape and were often elongated and parallel to the seed rows or to the direction of cultivation.

Table 1. Percentage disease severity in wheat and barley in the zones within bare patches and in control plants outside the patch.

Patch No.	Zone ¹						
	control	A	В	С			
1	6.4	40,0	63.3	80.0			
2	11.7	24.0	56.0	83.2			
3	6.3	40.0	64.3	83.4			
4	15.6	20.0	58.3	72.6			
5	41.7	50.0	62.9	79.6			
6	11.2	22.3	55,2	89.2			
7	28.8	23.1	48.8	88.6			
8	22.4	27.5	63.9	87.9			
9	19.4	45.2	56,1	92.3			
10	23.1	14.1	56.0	64.1			
11	13.9	33.3	70.2	84.0			
12	13.2	36.5	73.2	81.9			
Mean ²	17.8a	31.3b	60.7c	82.20			

 $^{^{1}}$ Zones: A = peripheral; C = central; B = intermediate zones within patches.

The plants were stunted and leaves were chlorotic. Roots were discoloured from light to dark brown with soft tips, and were often nipped off near the crown. Disease severity varied significantly among patch zones (Table 1), suggesting that plants at the centre (C) were more heavily diseased than those at the periphery and intermediate zones of A and B. Control plants outside the patches were affected as well although to a much less extent.

Fungal isolation and identification. The tissue transplant method yielded growth of different soil-borne and root-rot fungi, viz. Fusarium spp., Rhizoctonia spp., Mortierella spp., Bipolaris sorokiniana (Sacc.) Shoem., Pythium sp. and Trichoderma sp. (Table 2). Most fungi were isolated with a higher frequency on WA than on PDA. Isolates of Rhizoctonia spp. were identified following Parmeter et al's (1969) species-

Table 2. Fungi isolated from 12 bare patches of wheat and barley.

Fungi	Frequency of occurrence (%)	
Fusarium spp.	37.2	
Rhizoctonia spp.	27.0	
Mortierella spp.	21.5	
Bipolaris sorokiniana	7.1	
Pythium sp.	0.7	
Trichoderma sp.	6.3	

² Means with the same letter are not significant at 5% level (Duncan's Multiple Range Test).

Table 3. Measurements (in µm) of basidia, sterigmata and basidiospores of Thanatephorus cucumeris and Ceratobasidium spp. associated with bare patch of wheat and barley.

Species	Ag	Basidia			Sterigmata	æ		Basidiospores	es		
		length	width		length		width	length		width	
T. cucumeris	$2-1^{1}$	11.3	9.5		$8-11.5 (9.6)^2$		1.5-3 (2.3)				(7.7)
T. cucumeris	$2-2^{1}$	12.7	9.2		11.5-15.5 (13.1)		2.5-3.5 (2.7)	4.5-6.5		3-5 ((3.8)
T. cucumeris	6^1			(8.7)	5-24.5		1.5-3.5 (2.4)				(0.4
T. cucumeris	81	7-17 (12.9)	6.5-12.5	(6.7)	5-20	(11.1)	1.5-5.2 (2.8)	3-12.5			4.3)
T. cucumeris	(Warcup and										
	Talbot, 1962)	15-18	8-10		up to 5		3	6-7	7	4-6.3	
T. cucumeris	(Talbot, 1970)	10-25	6-19		5.5-36.5		3-4	6-14	7	8-1	
T. cucumeris	(Wong and Sivasi-										
	thamparam, 1985)										
	Ag 2-1	11.5-12.7 (16.6)		(8.1)	1.5-33.1 (14.1)		1.5-3.8 (2.4)	5.4-14.6 (8.)		.5-7.3 (5.1)
	Ag 2-2	8.5-20 (14)	4.2-9.6	(8.2)	3.1-28.5		1.5-4.2 (2.6)	4.6-13.1 (7.3		2.7-7.3	5.6)
Ceratobasidium sp.	$A_{ m g}$ $C^{ m l}$				16-30		1.5-3.5 (2.3)	6-8 (7.3)		3.5-5 (4.4)	4.4)
Ceratobasidium sp.	Ag C (Wong and										
	Sivasithamparam,										
	1985)	11.9-19.2 (16)	11.2-12.3 (11.7)		3.5-20		1.5-4.6 (3)		(£.	3.8-8.1 (5.7)	5.7)
Ceratobasidium sp.	unassigned ¹	10-12.5 (11)	7.5-9		7-13	(10.5)	1.5-3.5 (2.4)	4.5-9 (5.6)	· ·	5 ((3.9)
Ceratobasidium sp.	(Parmeter et al.,										
•	1967)	10.8-16.2	8.1-10.8		6.8-17.6		1.4-2.7	6.8-10.8	7	4.1-5.4	
	(Warcup and										
	Talbot, 1965)	13-17	9-12		7		ς.	6.5-9.3	7	4.5-6	

¹ Isolates from our investigation.
² Values in parentheses are the overall mean of isolates.

Table 4. Distribution of multinucleate and binucleate isolates of *Rhizoctonia* spp. in the three zones within bare patches of wheat and barley.

Nucleation type	Number p	er zone ¹			Total	
	control	A	В	С	number	070
Multinucleate	4	39	41	64	148	90
Binucleate	. 0	12	3	2	17	10
Total	4	51	44	. 66	165	100

¹ For explanation of zone types, see Table 1.

delineation (Table 3). One hundred and sixty-five isolates of *Rhizoctonia* spp. were obtained from 12 patches and control plants of wheat and barley. Ninety percent of these isolates were multinucleate and 10% were binucleate (Table 4). Numbers of multinucleate and binucleate isolates in the three zones within patches differed considerably. Multinucleate isolates were isolated mostly in zone C and less in zones B and A, unlike binucleate isolates which were isolated in relatively greater numbers in A and less in B and C.

After 3 to 4 weeks' growth on PDA at 25 °C, brown and yellowish colour types could be distinguished. The colour intensities of the brown isolates (predominantly multinucleate) ranged from pale to dark brown. Mycelium of pale brown isolates grew appressed to the PDA surface with little aerial growth, while that of dark brown isolates had denser aerial mycelium. Multinucleate isolates on PDA produced brown crusty sclerotia ranging from 0.15 mm to 0.5 mm in diameter and most grew superficially on the agar surface. The colour intensity of the yellow isolates (which were predominantly binucleate) ranged from pale yellow to maize yellow. These yellowish isolates produced little white aerial mycelium. Binucleate isolates did not produce sclerotia on PDA, WA or CMA. The growth rates of colonies were not related to the anastomosis groupings. Growth rate of multinucleate (14.5 to 22.3 mm per day) and binucleate (13.7 to 18.8 mm per day) isolates did not differ on PDA at 25 °C and therefore was not considered as a reliable criterion in separating isolates of Rhizoctonia spp. All multinucleate isolates were identified as R. solani and possessed 4 to 10 nuclei per cell, but nuclear number varied from cell to cell and from one isolate to another, while binucleate *Rhizoctonia* spp. predominantly had 2 nuclei per cell.

Anastomosis grouping. Of the 165 isolates of Rhizoctonia spp. examined (Table 5), six binucleate isolates were assigned to Ag C, D, E and K and 99 multinucleate isolate to Ag 2-1, 2-2, 5, 6, Bl and 8. Eleven binucleate and 49 multinucleate Rhizoctonia spp. were not assigned to any group.

Teleomorphic states. In basidia induction tests, three binucleate isolates (1 Ag C and 2 unassigned Ag) produced a teleomorphic state which resembled that of Ceratobasi-dium cornigerum (Bourd) Rogers (1935) as described by Warcup and Talbot (1962) and the Ceratobasidium sp. described by Parmeter et al. (1967). Thirty multinucleate isolates (3 Ag 2-2, 8 Ag 2-1, 3 Ag Bl and 16 Ag 8) produced the teleomorphic state which is typical of Thanatephorus cucumeris. Measurements of basidia, basidiospores,

Table 5. Characteristics of *Rhizoctonia* spp. isolated from wheat and barley plants from bare patches.

Species	Ag ¹	Number of isolates	Nuclear condition ²	Pigment- tation	Sclerotia ³
R. solani	2-1	27	MN	pale-dark brown	+
	2-2	20	MN	pale-dark brown	+
	5	2	MN	pale-dark brown	+
	6	1	MN	pale-dark brown	+
	Bl	2	MN	pale-dark brown	+
	8	40	MN	pale-dark brown	+
	* 4	49	MN	brown	+
Rhizoctonia spp.	C	2	BN	maize yellow	_
••	D	1	BN	maize yellow	
	E	2	BN	maize yellow	
	K	1	BN	maize yellow	
	* * 4	11	BN	maize yellow	

¹ Anastomosis grouping.

sterigmata and supporting hypha below basidium of our *T. cucumeris* isolates were similar to those of Rogers (1935), Warcup and Talbot (1962, 1965), Parmeter et al. (1967), Talbot (1970) and Wong and Sivasithamparam (1985), (Table 3).

Pathogenicity tests. Of the thirteen isolates tested, Ag 2-1, Ag 2-2 and Ag 8 were the most pathogenic, with Ag 8 being the most virulent to wheat. The binucleate isolates i.e. Ag D, E, K and the unassigned one were moderately to mildly pathogenic (Table 6).

Discussion

The results indicate that there are at least two different *Rhizoctonia* species and several anastomosis groups associated with bare patch of wheat and barley even within one paddock in a farm. Noteworthy as well is the marked difference in sizes and disease levels of plants sampled from the three zones and the presence of multinucleate isolates of *Rhizoctonia* spp. in plants taken outside the patch which were sampled as control plants. The area affected by the patch disease was variable, the size of some of the patches being larger than those recorded by MacNish (1985) who found that the mean area of the patches within three crops from four consecutive years ranged from 0.7 to 2.7 m².

There is a predominance of multinucleate isolates within the bare patches. This could be expected as *Thanatephorus cucumeris* has been implicated as the causal organism of bare patch. There was a sharp contrast in the frequency of occurrence of multinucleate and binucleate isolates of *Rhizoctonia* spp. in the three zones, with the multi-

² MN = multinucleate; BN = binucleate.

 $^{^{3}}$ + = sclerotia formed by all isolates; — = sclerotia not formed by any isolates.

^{4 *; ** =} unassigned multinucleate and binucleate isolates respectively.

Table 6. Root disease index of wheat inoculated with binucleate and multinucleate isolates of *Rhizoctonia* spp.

Isolate No.	Ag	Nuclear condition ¹	Treatment means ²
I-90	(Ag 2-1)	MN	80.32 a
I-22	(Ag 8)	MN	77.00 a
I-21	(Ag 2-2)	MN	76.20 a
I-56	(Ag 5)	MN	55.60 b
I-88	(Ag 2-1)	MN	21.60 c
I-84	(Ag Bl)	MN	17.80 cd
I-7	(Unassigned)	BN	13.20 cde
I-143	(Ag K)	BN	12.20 cde
I-161	(Ag E)	BN	10.80 cde
I-59	(Ag 6)	MN	10.74 cde
I-144	(Ag D)	BN	7.80 de
I-83	(Ag Bl)	MN	6.20 e
	Control		3.00 e

¹ MN = multinucleate: BN = binucleate.

nucleate isolates being predominant in zone C (centre of patch). The relative increase in activity of the binucleate isolates in zone A may be related to the abrupt edging of the patch at the periphery resulting from the competition of avirulent isolates with the multinucleate forms.

Without further work it may not be possible to disregard the relationship of associated organisms such as *Fusarium* spp. and *B. sorokiniana* with *Rhizoctonia* spp. in the development of the bare patch. Work by Moen and Harris (1985) has shown that a complex of organisms may be responsible for the Rhizoctonia disease complex of wheat and barley in South Australia.

The nuclear condition of vegetative hyphal cells was found to be very helpful in separating *R. solani* isolates from other *Rhizoctonia* spp. Binucleate isolates did not produce sclerotia and dark mycelial pigmentation on PDA. Burpee et al. (1980) obtained similar results and suggested that this characteristic should be a principal distinguishing feature in separating these isolates from *R. solani*.

Of the multinucleate *R. solani* isolated, the great majority were Ag 2-2, Ag 2-1 and Ag 8. All were highly pathogenic. The other Ag groups (Ag 5, 6 and Ag Bl) were less frequent and less pathogenic. Binucleate *Rhizoctonia* spp., which were relatively less frequent within the patches, were low in pathogenicity as well. All multinucleate pathogenic anastomosis groups encountered in our study belong to the teleomorph *T. cucumeris* and all binucleate ones which fruited were found to belong to *Ceratobasidium* sp.

R. solani Ag 2-1, Ag 3, Ag 4 and Ag 8 have been reported in South Australia (Neate, 1985; Neate and Warcup, 1985). Neate (1985) also reported the existence of Rhizoctonia spp. other than R. solani in South Australian wheatfields. These included Cera-

² Means with the same letter are not significantly different from each other (P < 0.01, Duncan's Multiple Range Test).

tobasidium cornigerum, Waitea circinata Warcup and Talbot and Iodophanus carneus (Pers.) Korf.

It would be interesting to examine the distribution and pathogenicity of potential fungal pathogens within bare patches of cereals occurring in Europe and the USA to compare them with those we have observed in Western Australia. Our report is the first indicating the presence within bare patches of multinucleate Ag 2-2, Ag 2-1, Ag 6, Ag 5 and Ag Bl and binucleate Ag C, D, E and K in Western Australia or anywhere else in Australia or overseas. Work is also needed to examine whether the dominance of multinucleate *Rhizoctonia* isolates in the centre of patches in early stages of patch formation is succeeded by the dominance of binucleate forms with the progression of disease. We also suggest that a complex of fungi may be involved in the production of bare patches, although individual isolates are capable of destroying roots on their own. Further work is currently underway to evaluate the effect of inoculation of wheat with various combinations of binucleate and multinucleate *Rhizoctonia* species in the presence or absence of other fungi which have been isolated from affected roots.

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Samenvatting

Identiteit en pathogeniteit van Rhizoctonia spp. van kale-plekkenziekte in granen op een perceel in West Australië

Van kiemplanten van tarwe en gerst, afkomstig van een met kale-plekkenziekte besmet perceel in West Australië werd de mate van wortelbeschadiging bepaald en werden schimmels uit de wortels geïsoleerd. De plekken waren verschillend van vorm en afmeting; de zwaarst aangetaste planten werden in het centrum ervan aangetroffen. Van de 165 verkregen isolaten van Rhizoctonia spp. was 90% meerkernig en 10% tweekernig. De meerkernige overheersten in de centra van de plekken. Relatief gezien nam het aantal tweekernige isolaten toe naarmate de herkomst dichter bij de periferie van de plekken was. De scherpe begrenzing van de ziekte aan de randen van de plekken zou in verband kunnen staan met het toenemen van de activiteit van de avirulente tweekernige isolaten in de nabijheid van de periferie van de plekken. Een aantal andere schimmels, zoals Fusarium spp., Mortierella spp., Bipolaris sorokiniana, Pythium sp. en Trichoderma sp. werd eveneens in de plekken aangetroffen. De meerkernige isolaten die tot de anastomosegroepen Ag 2-1, 2-2 en 8 (Thanatephorus cucumeris) behoren, waren voor tarwe het meest pathogeen. De tweekernige isolaten van de anastomosegroepen Ag C, D, E en K (Ceratobasidium sp.) waren minder pathogeen. Gesuggereerd wordt, dat de kale-plekkenziekte veroorzaakt wordt door een complex van verschillende wortelschimmels, die behoren tot een of meer anastomosegroepen van R. solani en andere daarmee geassocieerde schimmels.

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