Characterization of Anastomosis Group 11 (AG-11) of *Rhizoctonia solani*

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ABSTRACT

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Isolates of *Rhizoctonia solani* anastomosis group 11 (AG-11) were collected in Western Australia and Arkansas. Some isolates from each location fruited on 1.5% water agar, 2% V8-juice agar, and soil overlay plates and produced sexual structures typical of *Thanatephorus cucumeris*. Mycelium of isolates of AG-11 growing on potato-dextrose agar was white to light tan when young, but became brown to dark brown with age. Concentric rings of dark and light mycelium were visible in most cultures. Mature sclerotia were tan to light brown and were scattered over the agar surface. No anastomosis reactions (C0) were observed between paired isolates of AG-11 and anastomosis groups 1, 3–7, 9, and 10. C1 anastomosis reactions were observed between some isolates of AG-11 and some isolates of AG-2, -8, and -BI, indicating a distant or "bridging" anastomosis relationship with these three AG. Isolates of AG-11 were auxotrophic for thiamine. Isolates of AG-11 are known to damage lupine, soybean, and other plants in the field, and in greenhouse and growth chamber studies were shown to damage other crops, including cotton, radish, wheat, and potato.

Rhizoctonia solani Kühn (teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk) is a cosmopolitan plant-parasitic fungus that can attack a broad range of host plants. The species *R. solani* is not a discrete taxonomic unit but rather is a "collective species" (1) comprised of many related but apparently genetically isolated groups. Many different criteria have been used to delineate subgroups of *R. solani*. Earlier methods of subgrouping included differences in colony morphology, host range, virulence, and so on, whereas recent methods include biochemical and molecular techniques (21).

Perhaps the most widely used method of subdividing *R. solani* into smaller more homogeneous groups is based on hyphal anastomosis (6,14,18). Hyphal anastomosis in *R. solani* is a manifestation of somatic, or vegetative, incompatibility (1) between hyphae. Hyphae of isolates within the same anastomosis group (AG) anastomose with one another, whereas isolates from different AG do not. Eleven AG of *R. solani* have been reported, including groups 1–10 and BI (5,14,20). Many of these AG have been subdivided based on cultural, physiological, pathological, biochemical, and molecular characteristics (5,14,21). Grouping isolates of *R. solani* by anastomosis reaction or by anastomosis reaction in combination with other methods of grouping produces subunits of *R. solani* that are sometimes called intraspecific groups (ISG) (14).

Some isolates of *R. solani* collected in Western Australia and Arkansas recently were found to constitute what we believe to be an undescribed AG. Isolates from Western Australia cause hypocotyl rot in lupine and coleoptile rot in wheat and are members of zymogram group 3 (ZG3) (16,22–25). Isolates from Arkansas were collected from rice and soybean seedlings or soil. They cause lesions on the coleoptile of wheat and are pathogenic on several other species of crop plants (19). The populations from Western Australia and Arkansas are indistinguishable from one another in terms of anastomosis reactions and constitute a group that we propose to call AG-11. AG-11 is characterized in this report.

MATERIALS AND METHODS

Collection and isolation. Multiple tester isolates representing each of the described anastomosis groups of *R. solani* are listed in Table 1. All isolates of AG-11 from Western Australia (Table 2) were collected from infected lupine (*Lupinus angustifolius* L.) hypocotyl tissue from field plantings of lupine (17). Isolates of AG-11 from Arkansas (Table 2) were collected from soil and plants. For soil isolations, samples were placed with a multiple pellet soil sampler (8) onto petri plates containing the Ko and Hora medium (10), modified by substituting aluminum tris for fenaminosulf (7). Soil samples also were assayed with the beet (*Beta vulgaris* L.) seed baiting technique (12). Isolates from rice (*Oryza sativa* L.) and soybean (*Glycine max* (L.) Merr.) seedlings were collected by rinsing the seedlings in running tap water for 45 min followed by a treatment for 90 s in 0.5% sodium hypochlorite. Pieces of surface-disinfested tissue were then placed on 1.5% water agar amended with ampicillin and rifampicin at 250 and 10 mg/L, respectively.

AG determination. AG identities were determined according to a modification of the method of Parmeter et al (18), used by Carling et al (5). Anastomosis reactions observed were grouped into one of four categories ranging from C0 (no reaction) to C3 (the equivalent of self-anastomosis). Definitions of categories have been reported (3,15).

Perfect-stage determination. Attempts were made to induce isolates of AG-11 to sporulate on 1.5% water agar and 2% V8-juice agar (18 g of agar per liter plus 20 ml of V8 juice per liter) and by using Ogoshi's soil overlay technique (13).

Cultural characteristics. Isolates of *R. solani* AG-11 were grown on potato dextrose agar (PDA) at 20–21 C in the dark. Observations of cultural characteristics were made over a 3-wk period.

Hyphal diameter and nuclear number. Hyphal diameter and number of nuclei per cell were determined on cultures grown at 20–23 C on cellophane overlaying 1.5% water agar in petri plates. Mycelium was stained with 3% KOH and safranin O (2) and examined with bright field optics at 400×. Hyphal diameter was determined on each of ten isolates by measuring 20 cells per isolate at right angles to the longitudinal cell wall. Nuclei

TABLE 1. Tester isolates representing the 11 established anastomosis groups (AG) of *Rhizoctonia solani* used in this study

AG	Subgroup	Isolate	Origin	Host	Collector or supplier
1	IA	CSKa	Japan	*Oryza sativa*	S. Kuninaga
1	IB	SFBV-1	Japan	*Beta vulgaris*	S. Kuninaga
1	IC	M43	Quebec	*Pinus resinosa*	N. A. Anderson
2	1	B275	Alaska	*Hordeum vulgare*	D. E. Carling
2	1	F56L	Alaska	*Solanum tuberosum*	D. E. Carling
2	1	457	Japan	Unknown	E. E. Butler
2	1	90-27-7	Minnesota	*Brassica oleracea*	C. E. Windels
2	2IIIB	C330	Japan	*O. sativa*	A. Ogoshi
2	2IIIB	B60K	Japan	*B. vulgaris*	S. Kuninaga
2	2IV	RI64	Japan	*B. vulgaris*	A. Ogoshi
2	2IV	RH16	Japan	*B. vulgaris*	S. Kuninaga
3	...[a]	ST-11-6	Japan	*S. tuberosum*	A. Ogoshi
3	...	SCL24	Alaska	*S. tuberosum*	D. E. Carling
4	HG-I	AH-1	Japan	*Arachis hypopaea*	S. Kuninaga
4	HG-II	RR5-2	Japan	*B. vulgaris*	S. Kuninaga
4	?[b]	SF-1	Arkansas	*Gossypium hirsutum*	C. Rothrock
5	...	P99	North Dakota	*S. tuberosum*	R. W. Stack
5	...	ST-6-1	Japan	*S. tuberosum*	A. Ogoshi
5	...	W3 HTA-1	Japan	*Triticum aestivum*	A. Ogoshi
6	HG-I	NTA3-1	Japan	Soil	S. Kuninaga
6	GV	NKN2-1	Japan	Soil	S. Kuninaga
7	...	1535	Japan	Soil	S. Kuninaga
7	...	1556	Japan	Soil	S. Kuninaga
8	ZG1-1	89570	W. Aust.	*H. vulgare*	G. C. MacNish
8	ZG1-1	91784	W. Aust.	*Lupinus angustifolius*	G. C. MacNish
8	ZG1-2	90812	W. Aust.	*L. angustifolius*	G. C. MacNish
8	ZG1-2	SA50	S. Aust.	*Avena sativa*	A. Dubé
8	ZG1-2	CI	Washington	*H. vulgare*	E. N. Bassett
8	ZG1-3	SA1512	S. Aust.	Unknown	S. M. Neate
8	ZG1-4	88351	W. Aust.	*H. vulgare*	G. C. MacNish
8	ZG1-4	91006	W. Aust.	*H. vulgare*	G. C. MacNish
8	ZG1-5	91623	W. Aust.	*H. vulgare*	G. C. MacNish
8	ZG1-5	92547	W. Aust.	*H. vulgare*	G. C. MacNish
9	TP	V12M	Alaska	*S. tuberosum*	D. E. Carling
9	TX	S9R1	Alaska	Soil	D. E. Carling
10	...	W395	Washington	*T. aestivum*	A. Ogoshi
10	...	3751	Washington	*T. aestivum*	A. Ogoshi
BI	...	AI 1-4	Japan	Soil	S. Kuninaga
BI	...	TE 2-4	Japan	Soil	S. Kuninaga

[a]Subgroups either do not exist or have not been formally designated.
[b]Subgroup identity not known.

TABLE 2. Isolates of *Rhizoctonia solani* anastomosis group 11 from Arkansas (AR) and Western Australia (WA) used in this study

Isolate	Origin	Host	Collector or supplier
ROTH1	Colt, AR	Soil	C. S. Rothrock
ROTH3	Colt, AR	*Glycine max*	C. S. Rothrock
ROTH4	Colt, AR	*G. max*	C. S. Rothrock
ROTH5	Colt, AR	*G. max*	C. S. Rothrock
ROTH6	Colt, AR	*G. max*	C. S. Rothrock
ROTH7	Colt, AR	*G. max*	C. S. Rothrock
ROTH16	Colt, AR	*G. max*	C. S. Rothrock
ROTH19	Colt, AR	*G. max*	C. S. Rothrock
ROTH20	Colt, AR	*G. max*	C. S. Rothrock
ROTH24	Stuttgart, AR	*Oryza sativa*	C. S. Rothrock
ROTH25	Stuttgart, AR	*O. sativa*	C. S. Rothrock
ROTH26	Stuttgart, AR	*O. sativa*	C. S. Rothrock
R101	Dowerin, WA	*Lupinus angustifolius*	M. W. Sweetingham
R1013	Eradu, WA	*L. angustifolius*	M. W. Sweetingham
R1352	Wongan Hills, WA	*L. angustifolius*	M. W. Sweetingham
88292	Meredin, WA	*L. angustifolius*	G. C. MacNish
ZN56	Northampton, WA	*L. angustifolius*	W. J. MacLeod
ZN450	Northampton, WA	*L. angustifolius*	W. J. MacLeod
ZN667	Woorree, WA	*L. angustifolius*	W. J. MacLeod

were counted in 20 cells per isolate selected at random from a location in the stain gradient where nuclei and septa could be clearly observed.

Thiamine requirement. Petri dishes containing a Czapek-Dox solution broth (Difco Laboratories, Detroit) plus 2% purified agar (Difco), with or without 10^{-5} M thiamine hydrochloride, were seeded with 4-mm-diameter mycelial disks of selected isolates representing AG-11. Isolates representing AG-2-1 (prototrophic), AG-3 (prototrophic), and AG-5 (auxotrophic) were included for comparison.

Radial growth. Radial growth was determined by growing selected isolates representing 12 ISG of *R. solani* (including AG-1-IB, -2-1, -2-2, -4, -5, -6, -7, -8, -9, -BI, and -11) on PDA at 25 C in the dark. Radial growth was determined between 24 and 72 h after inoculation. Each treatment was replicated three times and the experiment was repeated once.

Virulence determination. Virulence of selected isolates of *R. solani* AG-11 was determined on cotton (*Gossypium hirsutum* L.), soybean, radish (*Raphanus sativus* L.), wheat (*Triticum aestivum* L.), rice, and potato (*Solanum tuberosum* L.). Virulence on potato was determined at 10 C according to the method used by Carling and Leiner (4). Virulence on plant species other than potato was evaluated by two methods. First, individual seeds of each crop were planted in 2.5-cm-diameter Conetainers (Stuewe and Sons, Inc., Corvallis, OR) that had been filled with sterile sand. Conetainers were placed in growth chambers at 25 C under 12 h of artificial light per day for 7–10 days. Disks of agar were cut from petri plates of PDA on which selected isolates of *R.*

solani were growing or from sterile PDA. Individual disks of this inoculum were placed just below the sand surface and adjacent to the hypocotyl or coleoptile of the seedlings (9). After inoculation, seedlings were returned to the growth chamber for 7 days then harvested. At harvest, seedlings were removed from the sand culture, washed in tap water, and graded according to the following scale: 0 = no symptoms, 1 = slight discoloration, 2 = moderate discoloration or small, nongirdling lesions, 3 = girdling lesions, and 4 = seedling dead. Each treatment was replicated six times, and the experiment was repeated three times. Second, sand-cornmeal-water (88:3:9) or silt loam soil-blended potato-water (81:10:9) inocula were prepared in Erlenmeyer flasks according to Ko and Hora (10). These mixtures were seeded with agar disks cut from actively growing cultures, incubated for 7 days, and used to infest previously autoclaved soil. Five seeds were planted in each 7.6 × 7.6-cm pot, and the pots were placed in the growth chamber. A pot was an experimental unit. Plant stands and disease symptom ratings were recorded after 3 wk. Each treatment was replicated four or five times depending on the experiment, and the experiment was repeated three times. Data from a representative experiment are presented. Analyses were conducted on disease ratings with the Kruskal-Wallis test, using SAS (SAS Institute, Cary, NC) because the categorical responses for the treatments did not have similar variation. This nonparametric test was used to avoid incorrect assumptions. Data analyzed were from individual rather than combined experiments.

RESULTS

Anastomosis reactions between AG-11 and other AG. Six representative isolates, three from Western Australia and three from Arkansas, were selected as tester isolates of *R. solani* AG-11. Each of these six isolates was then paired with tester isolates representing each of the 11 described AG, and anastomosis reactions were determined (Table 3). Tester isolates representing subgroups of the various AG were included whenever possible; thus, a total of 22 ISG of *R. solani* and AG-11 are included (Table 3).

All tester isolates representing anastomosis groups 1, 3–7, 9, and 10 gave C0 anastomosis reactions with all six tester isolates representing AG-11, indicating the absence of an anastomosis relationship between each of these AG and AG-11. The remaining three AG: AG-2, -8, and -BI each had at least one tester isolate that gave a C1 anastomosis reaction with at least one AG-11 tester. Of the three, AG-BI was least reactive, and AG-8 was most reactive with AG-11.

Of the 12 pairings between isolates of AG-BI and -11 (Table 3), only one gave a positive anastomosis reading and that reading was limited to a single C1 reaction. This is a positive though very weak anastomosis reaction. Of the 18 pairings between the six isolates of AG-11 and the three isolates of AG-2-1, nine gave C0 reactions, and nine gave C1 reactions. One isolate of AG-2-1 (90-27-7) produced a C1 reaction with all six AG-11 testers, a second (F56L) reacted with the three AG-11 isolates from Western

TABLE 3. Anastomosis reactions[a] between isolates of *Rhizoctonia solani* anastomosis group 11 (AG-11) from Arkansas and Western Australia and tester isolates representing the 11 established AG of *R. solani*

			Western Australia			Arkansas		
AG	Subgroup	Isolate	R1352	R101	ZN56	ROTH20	ROTH26	ROTH25
1	IA	CSKa	C0	C0	C0	C0	C0	C0
1	IB	SFBV-1	C0	C0	C0	C0	C0	C0
1	IC	M43	C0	C0	C0	C0	C0	C0
2	1	F56L	C1 (3)[b]	C1 (2)	C1 (2)	C0	C0	C0
2	1	457	C0	C0	C0	C0	C0	C0
2	1	90-27-7	C1	C1 (2)	C1 (1)	C1 (2)	C1 (3)	C1 (4)
2	2IIIB	C330	C0	C0	C0	C0	C0	C0
2	2IIIB	B60K	C0	C0	C0	C0	C0	C0
2	2IV	RI64	C0	C0	C0	C0	C0	C0
2	2IV	RH16	C0	C0	C0	C0	C0	C0
3	...[c]	ST-11-6	C0	C0	C0	C0	C0	C0
3	...	SCL24	C0	C0	C0	C0	C0	C0
4	HG-I	AH-1	C0	C0	C0	C0	C0	C0
4	HG-II	RR5-2	C0	C0	C0	C0	C0	C0
5	...	P99	C0	C0	C0	C0	C0	C0
5	...	ST-6-1	C0	C0	C0	C0	C0	C0
6	HG-I	NTA3-1	C0	C0	C0	C0	C0	C0
6	GV	NKN2-1	C0	C0	C0	C0	C0	C0
7	...	1535	C0	C0	C0	C0	C0	C0
7	...	1556	C0	C0	C0	C0	C0	C0
8	ZG1-1	89570	C1	C1 (3)	C1 (2)	C0	C0	C1 (2)
8	ZG1-1	91784	C1 (2)	C1 (4)	C1 (3)	C0	C1 (2)	C1
8	ZG1-2	90812	C1 (2)	C1 (1)	C1 (1)	C0	C0	C1
8	ZG1-2	SA50	C1 (1)	C1 (2)	C1 (2)	C1	C1 (2)	C1
8	ZG1-3	SA1512	C1	C1 (1)	C1	C1 (1)	C1 (2)	C1 (2)
8	ZG1-4	88351	C1 (2)	C1 (1)	C1 (3)	C0	C1 (1)	C1 (3)
8	ZG1-4	91006	C1 (3)	C1 (1)	C1 (2)	C0	C1 (4)	C0
8	ZG1-5	91623	C1 (2)	C1 (3)	C1 (2)	C0	C1 (1)	C1 (3)
8	ZG1-5	92547	C1	C1	C1 (3)	C1 (1)	C1 (1)	C1 (2)
9	TP	V12M	C0	C0	C0	C0	C0	C0
9	TX	S9R1	C0	C0	C0	C0	C0	C0
10	...	W395	C0	C0	C0	C0	C0	C0
10	...	3751	C0	C0	C0	C0	C0	C0
BI	...	AI 1-4	C0	C0	C0	C0	C0	C0
BI	...	TE 2-4	C1 (1)	C0	C0	C0	C0	C0

[a] Anastomosis reaction rating: C0 = no anastomosis reaction; C1 = hyphal contact and apparent connection of walls but no membrane to membrane contact.

[b] Numbers in parentheses indicate the number of C1 reactions observed. If no numbers in parentheses are listed, five or more C1 reactions were observed.

[c] Subgroups either do not exist or have not been formally designated.

TABLE 4. Anastomosis reactions[a] between tester isolates of *Rhizoctonia solani* anastomosis group 11 from Arkansas (AR) and Western Australia (WA)

Origin	Isolate	Isolate							
		R1352	R101	ZN56	ZN450	ROTH20	ROTH26	ROTH25	ROTH5
WA	R1352	C3	C2	C2	C2	C2	C2	C2	C2
WA	R101		C3	C2	C2	C2	C2	C2	C2
WA	ZN56			C3	C2	C2	C2	C2	C2
WA	ZN450				C3	C2	C2	C2	C2
AR	ROTH20					C3	C2	C2	C2
AR	ROTH26						C3	C2	C2
AR	ROTH25							C3	C2
AR	ROTH5								C3

[a] Anastomosis reaction rating: C2 = wall connection and pore plus death of anastomosing and adjacent cells; and C3 = fusion of wall and membrane.

TABLE 5. Dimensions of sexual structures of *Thanatephorus cucumeris* produced by isolates of *Rhizoctonia solani* anastomosis group 11[a]

Isolate	Origin[b]	Metabasidia		Sterigmata		Basidiospores	
		Length	Width	No./basidium	Length	Length	Width
ROTH19	AR	11.6 ± 0.4[c]	8.9 ± 0.3	3.9 ± 0.1	24.7 ± 1.4	8.2 ± 0.2	5.9 ± 0.1
		8.5–13.9[d]	7.7–10.8	3–4	18.5–40.8	6.9–9.2	4.6–6.9
ROTH24	AR	11.4 ± 0.4	8.8 ± 0.2	3.8 ± 0.1	19.0 ± 0.8	7.8 ± 0.2	5.2 ± 0.1
		7.7–15.4	6.9–10.8	3–4	14.6–26.2	6.9–9.2	4.6–6.2
ROTH16	AR	13.2 ± 0.5	8.7 ± 0.2	3.6 ± 0.1	26.5 ± 1.2	8.0 ± 0.2	6.0 ± 0.2
		11.6–19.3	7.7–10.8	3–4	15.4–38.5	6.9–9.2	4.6–6.9
R1013	WA	14.6 ± 0.4	9.5 ± 0.3	...[e]	26.3 ± 1.0	8.9 ± 0.2	6.3 ± 0.2
		11.6–17.7	8.5–13.1		17.7–34.7	7.7–10.0	5.4–7.7
R1352	WA	15.7 ± 0.5	10.6 ± 0.2	3.6 ± 0.1	26.1 ± 0.9	8.8 ± 0.3	6.3 ± 0.2
		11.6–20.0	9.2–13.1	2–4	22.3–37.7	5.4–10.8	4.6–7.7

[a] All measurements are in micrometers and are based on 20 observations per isolate.
[b] AR = Arkansas; WA = Western Australia.
[c] Mean ± standard error of the mean.
[d] Range.
[e] Data not taken.

TABLE 6. Diameter and number of nuclei in mature hyphal cells of isolates of *Rhizoctonia solani* anastomosis group 11 from Arkansas (AR) and Western Australia (WA)

Isolate	Origin	Hyphal diameter (μm)[a]		No. of nuclei per cell[a]	
		Mean[b]	Range	Mean[b]	Range
ROTH6	AR	8.6 ± 0.3	6.16–11.55	6.0 ± 0.3	4–9
ROTH3	AR	8.3 ± 0.2	6.93–10.01	6.0 ± 0.3	4–8
ROTH5	AR	8.7 ± 0.3	6.93–11.55	7.1 ± 0.4	4–11
ROTH7	AR	7.9 ± 0.3	5.39–10.01	6.4 ± 0.4	4–10
ROTH4	AR	8.2 ± 0.3	6.16–11.55	7.3 ± 0.4	5–11
R101	WA	8.1 ± 0.2	6.16–10.01	7.3 ± 0.5	4–11
88292	WA	7.9 ± 0.3	6.16–10.78	5.4 ± 0.3	4–8
ZN450	WA	7.8 ± 0.2	6.16–9.24	6.6 ± 0.6	4–14
R1352	WA	7.3 ± 0.2	6.16–9.24	8.1 ± 0.4	6–11
ZN667	WA	8.4 ± 0.2	6.93–10.78	7.3 ± 0.4	5–12

[a] Twenty observations per isolate.
[b] Mean values followed by standard error of mean.

Australia but not with the three from Arkansas, and a third (457) produced no reaction with any isolate of AG-11. All tester isolates of AG-2-2, including representatives of AG-2-2IIIB and AG-2-2IV, gave C0 anastomosis reactions with all six AG-11 testers. All tester isolates of AG-8 (Table 3) produced a C1 reaction with four or more of the six tester isolates of AG-11. Similarly, each tester isolate of AG-11 reacted with some of the nine AG-8 testers, although one (ROTH20) reacted in only three of the nine pairings. The Western Australian isolates of AG-11 were more reactive with AG-8 (27 positive anastomosis readings in 27 pairings) than were the AG-11 isolates from Arkansas (18 positive anastomosis readings in 27 pairings).

Anastomosis reactions among isolates of AG-11. Anastomosis reactions among isolates of AG-11 from Arkansas and Western Australia are summarized in Table 4. Four isolates from each of the two locations were paired in all possible combinations. Each isolate when paired with itself gave the expected C3 (or self) anastomosis reaction. Each isolate when paired with another isolate gave the C2 reaction, indicating membership in the same AG.

Perfect-stage development. Some isolates of AG-11 from Arkansas and Western Australia formed hymenia and fruited on 1.5% water agar, 2% V8-juice agar, and with the soil overlay method. Some isolates from Western Australia fruited especially well on soil overlay plates; however, many isolates from both locations (approximately 80% from Australia and 35% from Arkansas) could not be induced to fruit by any of these methods. Dimensions of metabasidia, sterigmata, and basidiospores produced on 2% water agar by five isolates (three from Arkansas and two from Western Australia) are presented in Table 5.

Cultural characteristics. Isolates of AG-11 grown on PDA were white to light tan when young but after 3 wk ranged from brown to dark brown. A few isolates had yellowish pigmentation. Concentric rings of dark and light mycelium were visible in most cultures, and this zonation was apparent from early stages of development. Mycelium was floccose in early stages of growth, but as cultures aged, mycelium became increasingly appressed to the agar surface. Sclerotia generally ranged from few to many and were 0.5 to 2.0 mm in size. Individual sclerotia often coalesced into large clumps. Mature sclerotia were tan to light brown and were scattered randomly over the agar surface. Cultural characteristics of isolates from Australia and Arkansas tended to be similar but Australian isolates were somewhat lighter in color.

Hyphal diameter and nuclear number. Mean diameters of mature hyphae of the 10 isolates examined ranged from 7.28 to 8.74 μm (Table 6). The mean number of nuclei in mature cells of the same 10 isolates ranged from 5.4 to 7.3 per cell (Table 6).

Thiamine requirement. The isolates of AG-2-1 and -3, representing thiamine prototrophic AG of *R. solani*, grew at approximately the same rate with or without thiamine (Table 7). The isolate of AG-5, representing thiamine auxotrophic AG, grew at approximately twice the rate (a growth ratio of 2.01) if thiamine was present in the medium. Growth ratios of the 10 evaluated

isolates of AG-11 (Table 7) ranged from 1.73 to 9.29, illustrating the thiamine auxotrophic nature of AG-11.

Radial growth. The mean radial growth rate at 25 C of 25 isolates of AG-11 was 17.3 mm and ranked slightly above the average of isolates of the 12 ISG evaluated for rate of growth.

Virulence determinations. A range of virulence on each host was observed among isolates of AG-11, and a range of susceptibility was observed among the host plants tested. This was true when inoculum was placed adjacent to the hypocotyl (or coleoptile) of the seedlings (Table 8) and when inoculum was mixed with the soil (Table 9). Cotton was the most susceptible of the hosts when inoculum was placed adjacent to the plant and when soil was infested with the isolates. One or more isolates of AG-11 caused disease on cotton and radish when hypocotyls were inoculated (Table 8). When soil was infested with isolates of AG-11, disease was produced by one or more isolates on cotton, radish, soybean, and wheat (Table 9). However, wheat and soybean showed very little damage. The isolate of AG-4 included for comparison was as or more aggressive than the isolates of AG-11 on each of the host plants tested. The isolates of AG-4 killed nearly all test plants when its inoculum was mixed with soil.

Virulence on cotton and wheat of single isolates of AG-11 and -8 was compared in a study in which inoculum was placed next to the hypocotyl or coleoptile (Table 10). More damage was caused on cotton by the isolate of AG-11, but the isolate of AG-8 on cotton, and both isolates on wheat, caused only slight discoloration. Virulence on potato of 21 isolates of AG-11 was compared with the virulence of isolates representing three other AG (Table 11). A minor amount of damage was caused to the developing sprouts of potato plants inoculated with AG-11, but essentially no damage was observed on the roots.

TABLE 7. Thiamine requirement of isolates of *Rhizoctonia solani* representing anastomosis groups 2-1, 3, 5, and 11

AG	Isolate	Origin[a]	Mycelial dry weight[b] (mg) Czapek (A)	Czapek + T[c] (B)	B/A
2-1	B275	AK	151.2	166.5	1.10
3	SCL24	AK	157.7	159.2	1.01
5	W3 HTA-1	Japan	104.4	210.0	2.01
11	ROTH3	AR	66.8	178.8	2.68
11	ROTH4	AR	59.3	180.7	3.05
11	ROTH5	AR	61.6	147.0	2.39
11	ROTH6	AR	81.7	178.8	2.19
11	ROTH7	AR	18.1	168.1	9.29
11	R101	WA	55.8	190.0	3.41
11	R1352	WA	22.6	174.8	7.73
11	ZN56	WA	104.8	181.3	1.73
11	ZN450	WA	99.5	201.0	2.01
11	ZN667	WA	22.1	126.3	5.71

[a] AK = Arkansas; WA = Western Australia.
[b] Mycelial dry weight figures are the average of at least three replications.
[c] Czapek-Dox medium amended with 10^{-5} M thiamine hydrochloride (T).

DISCUSSION

Isolates of *R. solani* AG-11 have been collected and studied in Western Australia for many years and are found in association with disease-damaged lupine, wheat, and other plant species

TABLE 8. Virulence of isolates of *Rhizoctonia solani* on cotton, soybean, radish, wheat, and rice[a]

Treatment	AG[b]	Disease severity index[c] Cotton	Soybean	Radish	Wheat	Rice
Control	–	0.2 ± 0.2[d]	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.3
Isolate						
ROTH6	11	2.8 ± 0.7	0.7 ± 0.2	1.7 ± 0.6	0.8 ± 0.2	0.5 ± 0.3
ROTH7	11	3.7 ± 0.3	0.5 ± 0.2	2.5 ± 0.5	1.0 ± 0.6	2.0 ± 0.7
ROTH5	11	1.8 ± 0.5	0.2 ± 0.2	1.2 ± 0.5	0.3 ± 0.2	2.0 ± 0.7
ROTH1	11	2.8 ± 0.4	0.0 ± 0.0	1.3 ± 0.3	1.0 ± 0.0	1.8 ± 0.5
SF1	4	4.0 ± 0.0	1.8 ± 0.2	2.8 ± 0.3	1.0 ± 0.0	1.8 ± 0.8
Kruskal-Wallis ($P = 0.05$)[e]		S	S	S	S	NS

[a] Inoculum placed on hypocotyl or coleoptile of developing seedlings.
[b] Anastomosis group.
[c] Disease index 0–4; 0 = no symptoms, 1 = slight discoloration, 2 = moderate discoloration or small lesions, 3 = girdling lesion, and 4 = seedling dead.
[d] Means of six replications followed by standard error of the mean.
[e] Mean scores calculated based on rank within a column were significantly (S) or not significantly (NS) different.

TABLE 9. Virulence of isolates of *Rhizoctonia solani* on cotton, soybean, radish, wheat, and rice[a]

Treatment	AG[b]	Disease severity index[c] Cotton	Soybean	Radish	Wheat	Rice
No inoculum	–	0.8 ± 0.3[d]	1.0 ± 0.4	0.2 ± 0.2	0.2 ± 0.2	0.0 ± 0.0
Autoclaved inoculum	–	1.0 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.2 ± 0.2	0.3 ± 0.2
Isolate						
ROTH6	11	3.3 ± 0.2	1.3 ± 0.2	2.1 ± 0.5	2.5 ± 0.3	0.9 ± 0.3
ROTH7	11	2.1 ± 0.7	0.5 ± 0.2	2.2 ± 0.4	1.4 ± 0.2	1.1 ± 0.3
ROTH5	11	3.0 ± 0.4	1.9 ± 0.4	2.2 ± 0.3	2.0 ± 0.1	1.4 ± 0.2
ROTH1	11	2.7 ± 0.3	1.4 ± 0.3	2.0 ± 0.2	2.2 ± 0.3	0.8 ± 0.3
SF1	4	4.0 ± 0.0	3.9 ± 0.1	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
Kruskal-Wallis ($P = 0.05$)[e]		S	S	S	S	S

[a] Autoclaved soil infested with 1% (w/w) chopped potato-soil inoculum.
[b] Anastomosis group.
[c] Disease index 0–4; 0 = no symptoms, 1 = slight discoloration, 2 = small lesions or moderate discoloration, 3 = girdling lesion, and 4 = seedling dead.
[d] Means of five replications followed by standard error of the mean.
[e] Mean scores calculated based on rank within a column were significantly (S) or not significantly (NS) different.

TABLE 10. Virulence of an isolate of *Rhizoctonia solani* anastomosis groups (AG)-8 and -11 on cotton and wheat

Treatment	AG	Disease severity index[a]	
		Cotton	Wheat
Control	–	0.0 ± 0.0[b]	0.1 ± 0.1
Isolate			
ROTH1	11	2.6 ± 0.3	0.9 ± 0.3
C1	8	0.2 ± 0.1	0.6 ± 0.3
Kruskal-Wallis ($P = 0.05$)[c]		S	NS

[a]Disease index 0–4; 0 = no symptoms, 1 = slight discoloration, 2 = moderate discoloration or small lesions, 3 = girdling lesion, and 4 = seedling dead.
[b]Means of 10 replications followed by standard error of mean.
[c]Mean scores calculated based on rank within a column were significantly (S) or not significantly (NS) different.

TABLE 11. Reaction of sprouts and roots of emerging potato plants to isolates representing several anastomosis groups (AG) of *Rhizoctonia solani*[a]

Treatment	No. of isolates	Damage assessment[b]	
		Sprouts	Roots
AG-7	8	0.03 ± 0.03[c]	0.00 ± 0.00
AG-8	2	0.70 ± 0.21	2.30 ± 0.15
AG-10	10	0.36 ± 0.07	0.04 ± 0.03
AG-11	21	1.10 ± 0.08	0.04 ± 0.02
Control	–	0.00 ± 0.00	0.00 ± 0.00
Kruskal-Wallis ($P = 0.05$)[d]		NS	S

[a]All isolates evaluated on potato cultivar Green Mountain.
[b]Damage assessment for sprouts and roots was made on a five-position scale: 0 = no damage, no lesions and 4 = all sprouts (or roots) dead.
[c]Means of five replications followed by standard error of the mean.
[d]Mean scores calculated based on rank within a column were significantly (S) or not significantly (NS) different.

(23,24). Isolates from Western Australia have been characterized as ZG3 (25), and more recently their AG affinity has been studied (16). MacNish et al (16) reported that isolates of ZG3 (AG-11) did not anastomose (C0 reaction) with tester isolates of most AG and produced a C1 anastomosis reaction with some isolates of AG-2-1 and AG-8, and they indicated their belief that ZG3 constituted an as yet unreported AG. Isolates of AG-11 from Arkansas have been found in association with rice and soybean seedlings and in an initial report (19) were described as potentially comprising a new anastomosis group. In the initial collections, 59% of the isolates of *R. solani* from soybean and 35% of those from rice were AG-11.

Our analysis of anastomosis reactions observed between isolates of AG-11, from Arkansas and Western Australia, and testers representing all other AG indicates no anastomosis reaction (C0) occurs between anastomosis groups 11 and 1, 3–7, 9, and 10. However, a limited or weak (C1) anastomosis reaction occurs between isolates of AG-11 and some testers representing AG-2, -8, and -BI. All subgroups of AG-8 reacted with isolates of AG-11, but within AG-2, only isolates of subgroup AG-2-1 reacted with isolates of AG-11.

The C1 reactions observed between tester isolates of AG-11 and tester isolates of AG-2-1, -8, and -BI indicate that a real but distant anastomosis relationship exists between AG-11 and these three AG. This anastomosis relationship may be similar to "bridging" relationships that are known to exist (in various combinations) between isolates of five AG: AG-2, -3, -6, -8, and -BI (11,14,20). The addition of AG-11 increases the number of AG with bridging capabilities to six. This bridging phenomenon was first described in conjunction with AG-BI (11); in fact, AG-BI was named the "bridging isolate" group because of the capability of its members to anastomose with isolates of many other AG.

Although isolates of AG-11 do anastomose in this limited way (bridge) with some members of three other AG, evidence supporting the creation of a separate group for AG-11 is convincing. For example, C2 anastomosis reactions are consistently observed, without regard to the geographic source, between paired isolates of AG-11, but isolates of AG-11 do not produce a C2 reaction when paired with isolates of any other AG. Additionally, isolates of the two AG most closely linked to AG-11 based on anastomosis reaction (AG-2 and -8) produce pectic isozyme patterns (zymograms) (16) that are clearly different from those produced by isolates of AG-11; to date, there are no known examples of unique ZG that are represented in more than one AG (16). Also, isolates of AG-11 are thiamine auxotrophic (Table 8), whereas AG-8 and -2-1 are thiamine prototrophic (14). Finally, isolates of AG-11 attack hypocotyl or coleoptile rather than root tissues and generally do not produce the bare patch symptom typically associated with isolates of AG-8 (16,23,24).

Isolates of AG-11 do not cause the bare patch symptom, but they are capable of causing damage to plants in the field, including hypocotyl rot in lupine and other legumes and coleoptile rot in wheat (22–24). Pathogenicity data included in this report indicate other plant species also are susceptible to infection and damage, including cotton, soybean, and radish, but additional studies will be needed to confirm this observation under field conditions.

At the present time, isolates of AG-11 are known to occur only in Western Australia and Arkansas, and anastomosis reactions between isolates representing these two populations indicate AG-11 is a comparatively homogeneous group. This homogeneity suggests there may be a relatively recent historic link between the two populations; however, we know of no such link at this time.

It is probable that the geographic distribution of AG-11 is much broader than is currently known and that subgroups exist. Additional studies of isolate virulence, host range, and relative susceptibility, as well as biochemical and molecular evaluations will be required to answer these questions. Indeed, isolates of AG-11 may provide material for some very useful studies of the taxonomy and distribution of *R. solani* by molecular biologists. Type isolates of AG-11 from Arkansas (ROTH25 and ROTH26) and Western Australia (R1352 and ZN56) are on file in the American Type Culture Collection in Beltsville, MD, as ATCC 90858, 90859, 90857, and 90860, respectively.

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