Comparison of Double-Stranded RNA Components and Virulence Among Isolates of *Rhizoctonia solani* AG-1 IA and AG-1 IB

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ABSTRACT

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Double-stranded RNA (dsRNA) was detected in 22 of 35 (63%) *Rhizoctonia solani* anastomosis group one (AG-1) intraspecific groups IA and IB isolates screened. Most AG-1 IA isolates had seven dsRNAs with molecular sizes ranging from 1.3 to 9.3 kb, whereas most AG-1 IB isolates had a 12-kb dsRNA. Not all isolates of AG-1 IA or IB obtained from the same soybean field had dsRNA. The dsRNAs were stable through six successive subculturings. Cell fractionation of *R. solani* revealed that dsRNAs were located in the microsomal fraction. The dsRNAs were no longer detected in two isolates of AG-1 IB and one isolate of AG-1 IA after 1 wk of growth at 35 C; however, the dsRNAs of one isolate of AG-1 IA were not lost at 35 C. The presence or absence of dsRNA in *R. solani* AG-1 IA or IB isolates did not correlate with virulence, mycelial growth, or phenol oxidase activity of the isolates. Extensive variation in the electrophoretic patterns of dsRNA was observed among the isolates of *R. solani* belonging to AG-1 IC, AGs-2, -3, -4, -5, and -7, and AG-BI. The dsRNA fingerprints of these anastomosis groups differed from those of AG-1 IA and IB.

Rhizoctonia foliar blight of soybean (*Glycine max* (L.) Merr.) in Louisiana is caused by isolates of *Rhizoctonia solani* Kühn belonging to anastomosis group one (AG-1), including AG-1 intraspecific groups IA (sheath blight subgroup), which causes aerial blight, and AG-1 IB, which causes web blight of soybeans (29). *R. solani* AG-1 IA and IB can be found in the same field (29) and sometimes on the same plant. A differential effect of temperature on the virulence of isolates of AG-1 IA and IB on soybean leaves and seedlings also has been reported (16). Isolates of AG-1 IA and IB are genetically different based on DNA-DNA homology (28). Recently, the genetic diversity of double-stranded (dsRNA) among isolates of *R. solani* in AGs-1–5 was demonstrated (3). The dsRNA components among isolates of AG-1 IA and IB also are genetically different (4). Double-stranded RNA in some isolates of *R. solani* has been reported to be viral in nature (9,25).

Castanho et al (5–7) reported the association of dsRNA with Rhizoctonia decline. They referred to this phenomenon as transmissible cytoplasmic hypovirulence, similar to that in *Cryphonectria parasitica* (2,8). Castanho et al (6) also suggested the use of the diseased isolate as a potential biocontrol agent. However, there is a lack of agreement on whether the dsRNA is involved in hypovirulence or virulence in *R. solani*. Finkler et al (9) reported that dsRNA was involved with virulence and not hypovirulence. Recent studies showed no significant correlation between virulence or hypovirulence and the presence of dsRNA (3). A direct or indirect correlation between specific dsRNA species and hypovirulence also has been suggested (3). Similarly, dsRNA is ubiquitous in natural populations of *R. solani* with no apparent correlation between the presence of dsRNA and the degree of pathogenicity (30). Recently, no significant correlation between the presence of dsRNA and pathogenicity was reported in *Diaporthe phaseolorum* var. *caulivora*, which causes stem canker of soybeans (17).

The presence or absence of different sizes and numbers of dsRNA molecules has been used to characterize field isolates of several fungal pathogens (20,26). Extensive intraspecific variation, which is correlated with the host range of the species (20), exists in the dsRNA banding patterns of *Puccinia* spp.

The present study was conducted to determine if variation in dsRNA components could be detected among the isolates of *R. solani* AG-1 IA and IB that are pathogenic to soybeans and to determine if any correlation exists between dsRNA and virulence. Additionally, we included the isolates of other anastomosis groups of *R. solani* (AGs-1–9 and AG-BI) in the study to compare dsRNA profiles.

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MATERIALS AND METHODS

Isolates and growth conditions. Information on *R. solani* isolates used in this study is presented in Table 1. Twelve isolates with the prefix BHIA (Ben Hur IA) or BH, belonging to the intraspecific group AG-1 IA and 14 isolates with the prefix BHMS (Ben Hur microsclerotial) or SMI (single microsclerotial isolates) belonging to the intraspecific group AG-1 IB also were included in this study. These were isolated from a soybean field at the Ben Hur Research Farm, Baton Rouge, LA. Cultures were maintained on potato-dextrose agar (PDA) and preserved at 4 C. For extraction of dsRNA, cultures were grown at room temperature (approximately 25 ± 2 C) in 200-ml Erlenmeyer flasks containing 50 ml of potato-dextrose broth (PDB). After 2 wk of growth, mycelial mats were harvested by vacuum filtration on Whatman No. 1 filter paper and freeze-dried for 24 h.

Extraction and purification of dsRNA. Double-stranded RNA was extracted from the freeze-dried mycelial mats with the method of Morris and Dodds (18) as modified by Valverde et al (27). All the samples were subjected to two cycles of cellulose (Whatman CF-11, Whatman, Clifton, NJ) chromatography. DNase and RNase treatment of the representative samples was done to confirm the dsRNA nature of the molecules, using previously described methods (12).

All dsRNA samples were subjected to polyacrylamide gel electrophoresis. Aliquots of 50 μl were loaded on 6% polyacrylamide gels (40:1, acrylamide/bisacrylamide) in a vertical slab gel apparatus. Electrophoresis was conducted at a constant voltage of 100 V for 3 h. The molecular size markers were dsRNAs of tobacco mosaic virus (6.5 kb), cucumber mosaic virus and its satellite (3.0 and 0.3 kb), bell pepper dsRNA (12 kb), dsRNA isolated by Lee et al (17) from *D. p.* var. *caulivora* (STJ-2 4.5, 1.8, and 1.4 kb), and *Penicillium stoloniferum* (1.4 kb). Gels were stained with ethidium bromide (20 ng/ml) for 30 min and photographed with Polaroid film 55 under UV light.

Stability of dsRNA. Isolates Crowley-2 and BHIA-14 were serially transferred up to six generations on PDA, and the subcultures obtained from each generation were subjected to dsRNA analysis. Isolates Crowley-2, BHIA-14, SMI-918, and Shiba-2 were grown at 35 C for 1 wk on PDA, and one agar plug (3 mm in diameter) from the growing edge of these colonies was transferred to 50 ml of PDB in 250-ml Erlenmeyer flasks. The flasks were incubated at room temperature for 2 wk. Freeze-dried mycelium of these isolates was subjected to dsRNA analysis as described previously.

Location of dsRNAs in cell. To determine the location of the dsRNA in the fungal cell, cell fractionation was done per the method of Kim and Klassen (14). Freeze-dried mycelium was ground in a mortar and pestle with extraction buffer (50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.44 M sucrose). This homogenate was centrifuged at 1,500 *g* for 5 min, and the pellet, composed of cell debris, was saved. The aqueous phase was centrifuged again at 3,500 *g* for 10 min, and the pellet was saved (nuclear fraction). The aqueous phase obtained after the second centrifugation was recentrifuged at 20,000 *g* for 10 min. The pellet obtained was the mitochondrial fraction, and the aqueous phase was the microsomal fraction. Each of these cell fractions was subjected to dsRNA extraction and analysis.

Virulence of isolates. To determine the virulence of isolates, detached leaflets of soybean cultivar Braxton, were inoculated as previously described (15,16,19). One agar plug (3 mm in diameter) of each of the isolates grown on PDA was used to inoculate each individual leaflet. Inoculated leaflets were incubated at room temperature (25–27 C). Data on disease severity (percent) per leaflet were recorded 72 h after inoculation for each isolate. The experiment was conducted twice, and the pooled data of the two experiments were analyzed with the SAS GLM procedure (24) because the results followed a similar trend and the variances were homogenous. In addition, the isolates grown at 35 C also were tested for virulence.

Mycelial growth. The isolates of *R. solani* used in this study were grown on PDA plates for 3 days. Agar plugs (3 mm) from the growing edge of cultures were placed in the center of 15-cm-diameter PDA plates and incubated at room temperature in the dark. The diameter (centimeters) of the colonies was measured 72 h after subculturing, using the agar plug as the center. The experiment had four replications and was conducted three times. The data from the three experiments were pooled because the results of the experiments followed a similar trend and the variances were homogenous. The data were subjected to statistical analysis with the SAS GLM procedure (24).

Phenol oxidase test. Agar plugs (3 mm in diameter) from the growing edge of the cultures of the various isolates on PDA were transferred onto plates of Bavendamm's medium (0.5% tannic acid, 1.5% Difco malt extract, 2% Difco bacto agar, pH 4.5). The plates were incubated at room temperature in the dark. Data on color reaction was recorded 1 wk after incubation as described by previous workers (17). The color reaction was scored as 0 = no color reaction; 1 = weak reaction; 2 = intermediate reaction; 3 = strong reaction; and 4 = very strong color reaction. This study was conducted twice with four replications each time. Pooled data of the two experiments were analyzed with the SAS GLM procedure (24).

TABLE 1. *Rhizoctonia solani* isolates[a] included in this study, anastomosis group (AG), geographic origin, source/host, and provider/collector

Isolate/AG[b]	Origin	Source/host	Provider/collector
R. solani			
1RS/1-IA	La.	Soybean	R. Vilgalys
Crowley-2/1-IA	La.	Soybean	C. S. Kousik
Waterway-1/1-IA	La.	Soybean	X. B. Yang
LA-2/1-IA	La.	Soybean	X. B. Yang
RK-1/1-IA	Miss.	Soybean	G. L. Sciumbato
RK-3/1-IA	Miss.	Soybean	G. L. Sciumbato
34RS/1-IB	D.C.	*Poa* sp.	R. Vilgalys
36RS/1-IB	Pa.	*Poa* sp.	R. Vilgalys
Shiba-2/1-IB	Japan	...[c]	M. C. Rush
46RS/1-?[d]	...	*Brassica* sp.	R. Vilgalys
3RS/1-IC	Canada	Norway pine	R. Vilgalys
13RS/1-IC	Japan	Soil	R. Vilgalys
SD-1/1-IC	S.Dak.	...	M. L. Carson
327/1-IC	N.Y.	Green bean	H. R. Dillard
322/1-IC	N.Y.	Green bean	H. R. Dillard
BV/1-IC	Japan	Beet	M. C. Rush
8RS/2-1	Austraia	Soil	R. Vilgalys
9RS/2-2	Minn.	Carrot	R. Vilgalys
C-116S/2-2 III	Japan	...	M. C. Rush
SD-2/2-2	S.Dak.	...	M. L. Carson
4RS/3	...	Green bean	R. Vilgalys
ST-11-6/3	Japan	Potato	M. C. Rush
7RS/4 HG-1	Minn.	...	R. Vilgalys
ATCC-18184/4	La.	Cotton	A. Shankarlingam
RLA-AG4/4	La.	Cotton	A. Shankarlingam
FLA-AG4/4	La.	Cotton	A. Shankarlingam
10RS/5	Japan	Soybean	R. Vilgalys
ST-6-1/5	Japan	Potato	M. C. Rush
72RS/6 HG-1	Japan	Soil	R. Vilgalys
74RS/6 GV	Japan	Soil	R. Vilgalys
76RS/7	Japan	Soil	R. Vilgalys
33RS/8	Scotland	*Hordeum* sp.	R. Vilgalys
72/8	Australia	Clover root	M. C. Rush
116RS/9	Ark.	Potato	R. Vilgalys
AG-9/9	...	...	G. E. Holcomb
22RS/BI	Japan	Soil	R. Vilgalys
TS-2-4S/BI	Japan	Soil	M. C. Rush
R. oryzae/...	Miss.	Rice	G. L. Sciumbato

[a] The study also included 12 *R. solani* isolates with the prefix BHIA or BH (AG-1 IA) and 14 isolates with BHMS or SMI (AG-1 IB), all isolated from a soybean field at the Ben Hur Research Farm, Baton Rouge, LA.

[b] Isolates of some anastomosis groups are subdivided into intraspecific groups. Details of these designations have been presented by Ogoshi (21).

[c] Not known.

[d] Intraspecific group has not been determined.

RESULTS

Double-stranded RNA was detected in 63% of the AG-1 IA and IB isolates. Sixty-one percent of the AG-1 IA isolates and 65% of the AG-1 IB isolates had dsRNA. Molecular sizes of the dsRNAs ranged from 1.3 to 9.3 kb in AG-1 IA isolates and from 1.5 to 12 kb in AG-1 IB isolates. Seven of the AG-1 IA isolates had seven dsRNA components (Table 2). Isolate Crowley-2 had only two (1.5 and 1.3 kb), and isolate Waterway had three dsRNAs (9.3, 1.6, and 1.26 kb). In AG-1 IB, 91% of the isolates with dsRNA had large dsRNAs (>4.5 kb), and of these, 73% had only a 12-kb dsRNA. Details of the dsRNAs in various isolates are presented in Table 2 and Figure 1. No significant differences were detected among the isolates of AG-1 IA or IB with respect to disease severity, mycelial growth, or phenol oxidase activity (Table 2). Contrasts between the means of dsRNA-containing and dsRNA-free isolates of AG-1 IA and IB indicated no significant differences for disease severity, mycelial growth, or phenol oxidase activity (Table 3). Isolates of the other anastomosis groups of *R. solani* also had dsRNAs; of the 30 isolates tested, 12 had dsRNAs. The number and sizes of the molecules varied among the isolates (Table 4; Fig. 2).

After growth at 35 C for 1 wk, dsRNAs could not be detected in isolates Shiba-2, SMI-918, and BHIA-14; however, the dsRNAs of isolate Crowley-2 appeared to be stable (Fig. 3). No significant difference in the virulence of the isolates grown at 35 C (without dsRNA) and those grown continuously at room temperature (with dsRNA) was noted (Table 5).

Serial subculturing of two isolates (BHIA-14 and Crowley-2) up to six transfers indicated that the dsRNA components were

TABLE 2. Disease severity on soybean leaves, mycelial growth, phenol oxidase activity, and double-stranded RNA (dsRNA) components of isolates of *Rhizoctonia solani* anastomosis group one (AG-1) intraspecific groups IA and IB

Isolate	dsRNA components (kb)	Disease severity/ leaflet[a]	Mycelial growth (cm)[b]	Phenol oxidase activity[c]
AG-1 IA				
LA-2	...[d]	82.5	14.0	1.5
BH-56	9.3, 7.8, 2.9, 2.1, 1.5, 1.4, 1.3	88.5	12.8	1.5
BHIA-14	9.3, 7.8, 2.9, 2.1, 1.5, 1.4, 1.3	95.5	14.0	1.9
BH-51	...	78.0	13.7	1.5
RK-1	...	94.5	14.1	2.1
BH57	2.9, 2.1,1.5, 1.4, 1.3	90.0	12.9	2.8
BH52	9.3, 7.8, 2.9, 2.1, 1.5, 1.4, 1.3	96.5	13.1	1.5
BH50	9.3, 7.8, 2.9, 2.1, 1.5, 1.4, 1.3	81.0	14.1	2.5
BH53	9.3, 7.8, 2.9, 2.1, 1.5, 1.4, 1.3	86.5	12.9	—[e]
Waterway	9.3, 1.6, 1.26	93.5	12.9	1.6
RK-3	...	99.5	13.9	1.9
BHIA-7	9.3, 7.8, 2.9, 2.1, 1.5, 1.4, 1.3	96.0	13.5	2.3
BHIA-1	...	85.0	—	1.8
BHIA-3	...	92.0	13.8	2.3
Crowley-2	1.5, 1.3	96.5	12.9	2.3
1RS	...	79.5	13.0	2.0
BHIA-10	9.3, 2.1, 1.5	94.0	13.3	1.3
BH-62	9.3, 7.8, 2.9, 2.1, 1.5, 1.4, 1.3	80.0	13.9	2.8
AG-1 IB				
BHMS-91-1	12	70.5	8.1	1.9
BHMS-91-7	12	79.0	8.7	2.8
SMI-915	12	67.0	10.7	2.0
BHMS-12	...	67.0	10.1	—
SMI-918	9.4, 2.1, 1.5	73.0	10.7	1.6
SMI-914	12	76.0	8.5	2.0
SMI-913	12	72.0	10.3	1.0
BHMS-91-3	12	66.5	10.0	2.0
SMI-912	12	75.0	9.8	1.0
SMI-917	12	63.5	10.4	1.6
BHMS-1	...	77.0	10.7	1.6
Shiba-2	9.5, 6.5	97.5	12.1	2.8
BHMS-91-2	...	75.0	—	2.8
36RS	...	—	11.0	3.0
34RS	9.4, 2.1, 1.5	89.0	11.2	1.4
BHMS-3	...	71.0	10.9	—
BHMS-2	...	81.5	8.6	—
$LSD_{0.05}$[f]		22.5	2.6	1.7

[a] Disease severity (percentage of leaflet area diseased) was measured on inoculated soybean leaflets of cultivar Braxton. Composite means of two experiments are presented.
[b] Mycelial growth was measured as colony diameter (centimeters) on potato-dextrose agar plates 72 h after inoculation. Composite means of three experiments are presented.
[c] Phenol oxidase activity of each isolate was measured in Bavendamm's medium based on color reaction on a rating scale of 0–4 in which 0 = no reaction and 4 = very strong color reaction.
[d] No dsRNA detected.
[e] Not tested.
[f] AG-1 IA and IB.

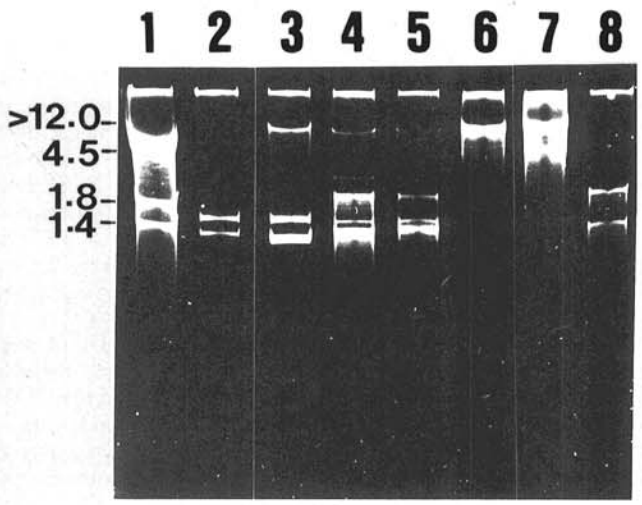

Fig. 1. Fractionation of double-stranded RNAs (dsRNAs) from isolates of *Rhizoctonia solani* AG-1 IA, IB, and IC on 6% polyacrylamide gels stained with ethidium bromide. Lane 1, dsRNA from *Diaporthe phaseolorum* var. *caulivora* (STJ-2); lanes 2–5, dsRNA from AG-1 IA isolates Crowley-2, Waterway, BH-52, and BH-50, respectively; lanes 6 and 7, dsRNA from AG-1 IB isolates SMI-913 and Shiba-2, respectively; lane 8, 322 (AG-1 IC). The numbers on the left indicate the sizes (kilobases) of the dsRNA standards.

TABLE 3. Contrasts between means of double-stranded RNA (dsRNA)-containing and dsRNA-free isolates of *Rhizoctonia solani* anastomosis group one (AG-1) intraspecific groups IA and IB

Variable	With versus without dsRNA	
	F	$P > F$[a]
AG-1 IA		
Disease severity[b]	1.08	0.3064
Mycelial growth[c]	3.02	0.0887
Phenol oxidase activity[d]	0.21	0.6470
AG-1 IB		
Disease severity	3.23	0.0831
Mycelial growth	0.54	0.4671
Phenol oxidase activity	0.95	0.3404

[a] Probability of obtaining a larger absolute value of *F*.
[b] Virulence of isolates was measured on the basis of disease severity (percent) per inoculated soybean leaflet of cultivar Braxton.
[c] Colony diameter (centimeters) was measured 72 h after transferring a mycelial disk to potato-dextrose agar in 15-cm-diameter petri plates.
[d] Phenol oxidase activity was determined in Bavendamm's medium based on color reaction on a rating scale of 0–4 in which 0 = no reaction and 4 = very strong color reaction.

very stable. The dsRNA components were present in the microsomal fractions of isolates BHIA-14 and Crowley-2. No dsRNA was detected in the nuclear and mitochondrial fractions.

DISCUSSION

About 54% (35 of 65) of all the isolates of *R. solani* tested contained dsRNA. Some of the isolates of AG-1 IA or IB obtained from the same field did not have dsRNA, indicating that not all the isolates of *R. solani* from the same field contain dsRNA. Castanho et al (7) reported that only three of 13 isolates of AG-1 contained dsRNA; however, Zanzinger et al (30) reported that dsRNA was common in a given population of field isolates of *R. solani*. In their study, 49 of 50 isolates of *R. solani* isolated from potato fields and belonging to five anastomosis groups (AGs-1–5) contained dsRNA. In other studies with *R. solani*, all isolates examined contained dsRNA (3,4). Similarly, the spores of all 26 isolates of *Puccinia* examined contained dsRNA (20). Tooley et al (26) reported that 36% of Mexican isolates of *Phytophthora infestans* contained dsRNAs, whereas dsRNA was not detected in any of the 20 isolates from the United States or Europe.

The fact that *R. solani* AG-1 IA and IB belong to the same anastomosis group, but different intraspecific groups (21), also has been demonstrated by DNA-DNA hybridization (28). Differences in dsRNA components also were detected between the two intraspecific groups. Most of the soybean isolates of AG-1 IB that had dsRNA contained only the 12-kb dsRNA, whereas 81% of the AG-1 IA isolates contained 9.3- and 7.8-kb dsRNAs. The 7.8-kb dsRNA was not found in the isolates of AG-1 IB isolated from soybeans, and the 12-kb dsRNA component found in the AG-1 IB isolates was not detected in any of the AG-1 IA isolates. However, many of the isolates of AG-1 IA and IB used in this study were isolated from a single field at the Ben Hur Research

TABLE 4. Mycelial growth, phenol oxidase activity, and molecular size of double-stranded RNA (dsRNA) components of isolates of *Rhizoctonia solani* belonging to various anastomosis groups (AG)

Isolate	dsRNA components (kb)	Mycelial growth (cm)[a]	Phenol oxidase activity[b]
AG-1 IC			
327	...[c]	14.2	1.8
322	2.1, 1.6	12.6	2.0
SD-1	...	13.0	2.4
13RS	...	14.2	2.6
3RS	...	13.5	3.1
BV	...	14.2	1.8
AG-2			
8RS	>12, 1.8, 1.3, 0.46	5.0	2.0
9RS	4.5, 2.7, 2.3, 1.4	7.1	3.0
AG-2-2SD	9.5, 7.4	7.4	2.1
C1165	...	6.0	1.2
AG-3			
4RS	9.4	5.3	1.0
ST-11-6	>12, 9.4, 7.3, 2.4, 1.8, 1.3	2.9	1.5
AG-4			
7RS	...	—[d]	2.0
ATCC-18184	7.3, 1.5	9.4	2.5
RLA-AG4	8.1, 3.2, 2.1, 1.5, 1.4, 1.3	8.7	—[d]
FLA-AG4	8.1, 3.2, 1.5, 1.4, 1.3	10.3	—
AG-5			
10RS	...	10.4	2.6
ST-6-1	9.4, 2.0, 1.7	9.4	3.0
AG-6			
72RS	...	—	3.0
74RS	...	6.8	3.4
AG-7			
76RS	>12	12.2	2.6
AG-8			
33RS	...	3.8	1.8
72	...	4.4	1.0
AG-9			
116RS	...	4.2	1.4
AG-9	...	3.5	1.0
AG-BI			
TS-2-4S	2.2	5.6	2.6
22RS	2.2	4.2	1.8
Others			
46RS (AG-1?)	...	13.9	1.8
R. oryzae	...	10.1	1.3
$LSD_{0.05}$		1.7	1.3

[a] Mycelial growth was measured as colony diameter (centimeters) on potato-dextrose agar plates 72 h after inoculation. Composite means of three experiments are presented.
[b] Phenol oxidase activity of each isolate was measured in Bavendamm's medium based on color reaction on a rating scale of 0–4 in which 0 = no reaction and 4 = very strong color reaction.
[c] No dsRNA detected.
[d] Not tested.

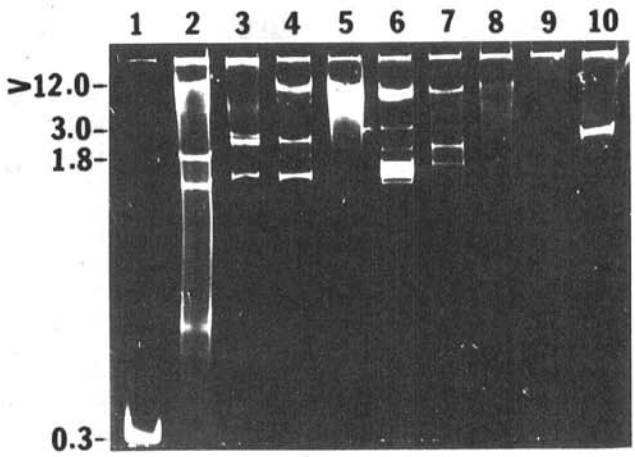

Fig. 2. Fractionation of double-stranded RNA (dsRNA) from isolates of *Rhizoctonia solani* on 6% polyacrylamide gels. Lane 1, Cucumber mosaic virus and its satellite; lane 2, 8RS (AG-2-1); lane 3, 9RS (AG-2-2); lane 4, ST-11-6 (AG-3); lane 5, 4RS (AG-3); lane 6, RLA-AG4 (AG-4); lane 7, ST-6-1 (AG-5); lane 8, 76RS (AG-7); lane 9, 33RS (AG-8); and lane 10, TS-2-4S (AG-BI). The numbers on the left indicate the sizes (kilobases) of the dsRNA standards.

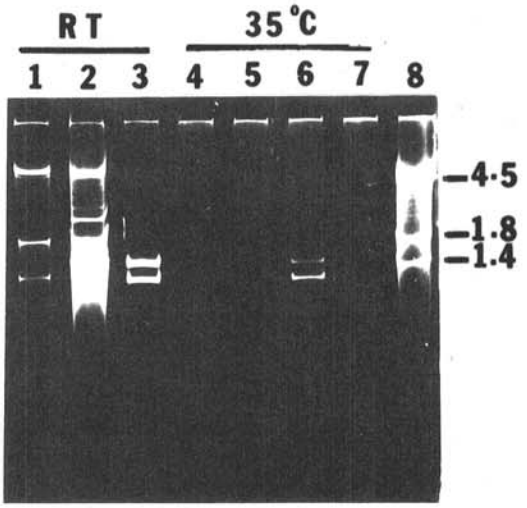

Fig. 3. Polyacrylamide gel (6%) electrophoresis of double-stranded RNA (dsRNA) from isolates of *Rhizoctonia solani* AG-1 IA and IB grown at room temperature and at 35 C. Lanes 1–3 contain isolates SMI-918, BHIA-14, and Crowley-2, respectively, grown at room temperature (25–27 C). Lanes 4–7, contain isolates SMI-918, BHIA-14, Crowley-2, and Shiba-2, respectively, grown at 35 C for 1 wk, subcultured, and grown at room temperature. Lane 8, dsRNA from isolate STJ-2 of *Diaporthe phaseolorum* var. *caulivora*. The numbers on the right indicate the sizes (kilobases) of the dsRNA standards (STJ-2).

Farm. Moreover, both of these isolates cause Rhizoctonia foliar blight of soybean and can be found in the same field (29) and sometimes on the same plant. These results suggest that the frequency of anastomosis between isolates of AG-1 IA and IB in the field is low, or there could possibly be some barrier to the transfer of dsRNA between AG-1 IA and IB. Recently, Bharathan and Tavantzis (4) reported that the dsRNAs from isolates of AG-1 IA and IB did not share sequence homology with the dsRNAs from the isolates of AG-1 IC. Similarly, it is possible that the dsRNA from the isolates of AG-1 IA and IB do not share sequence homology. However, further studies need to be done to determine this. Newton et al (20) reported that extensive intraspecific variation exists in the dsRNA patterns from *Puccinia* spp., and this variation is correlated with the host range of the species. They also suggested that such intraspecific variation in dsRNA offers a potential means of identifying intraspecific strains of fungi.

The isolates of AG-1 IB with the prefix SMI were isolated from single microsclerotia from soybean leaves and contained the 12-kb dsRNA. These results suggest dsRNA is present in the monilioid cells of the microsclerotia, and stable passage of the dsRNA occurs through the microsclerotia.

Castanho and Butler (5) reported that high-temperature (30 C) treatments did not have any curative effect on a diseased isolate of *R. solani* that contained dsRNA (7). Similarly, in experiments conducted on *D. p.* var. *caulivora*, the dsRNA was stable after a high-temperature treatment of 30 C (17). We observed that the dsRNA components in isolates of Shiba-2, SMI-918 (AG-1 IB), and BHIA-14 (AG-1 IA) were not detectable when the isolates were grown at 35 C. However, the dsRNA components were stable in isolate Crowley-2 after the same treatment. In our experiments, cultures were incubated at 35 C, because in our previous studies (16) we observed that the isolates of AG-1 IA infected soybean leaves at 35 C and the isolates also grew at this temperature. In spite of the loss of the dsRNA components in three of the isolates, there was no significant difference in the virulence of these isolates either with or without the dsRNA. However, in experiments conducted by Castanho et al (5–7) hyphal tip cultures of *R. solani* that did not contain the dsRNA grew better and were not diseased in comparison to the isolate containing dsRNA (5–7). In *C. parasitica*, eliminating the dsRNA in hypovirulent strains caused a dramatic increase in virulence (10).

The dsRNAs appeared to be very stable in *R. solani* through successive subculturing. Moreover, the isolates Crowley-2 and BHIA-14 had been regularly subcultured for more than a year before these experiments were conducted. Similarly, the dsRNA of *C. parasitica* was stable through serial transfers up to seven transfers (1) and through five transfers in *D. p.* var. *caulivora* (17).

TABLE 5. Virulence of isolates of *Rhizoctonia solani* anastomosis group one (AG-1) intraspecific groups IA and IB with or without double-stranded RNA (dsRNA) components on leaflets of two soybean cultivars

	Disease severity/leaflet[a]			
	Braxton		Davis	
Isolate	RT[b]	35 C[c]	RT	35 C
AG-1 IA				
BHIA-14	99	99	81	87
Crowley-2	85	88	75	81
AG-1 IB				
Shiba-2	91	88	81	87
SMI-918	91	94	65	75

[a] Virulence of isolates was measured as disease severity (percentage of leaflet area diseased) per inoculated soybean leaflet of cultivar Braxton grown in a greenhouse and on leaflets of cultivar Davis obtained from soybean plots at Ben Hur Research Farm, Baton Rouge, LA.

[b] RT indicates isolates grown continuously at room temperature. These isolates had all the dsRNA components.

[c] Isolates were grown on potato-dextrose agar at 35 C for 1 wk, subcultured onto potato-dextrose broth, grown for 2 wk, and analyzed for dsRNA. No dsRNA was detected in isolates of BHIA-14, SMI-918, and Shiba-2 after growth at 35 C for 1 wk.

Castanho et al (7) referred to the dsRNA in *R. solani* as the transmissible cytoplasmic hypovirulence. Similarly, dsRNA was detected in the cytoplasm in the case of *D. p.* var. *caulivora* (17). However, in *C. parasitica* the dsRNA was packed in fungal vesicles (11) and was found in mitochondria in *Ceratocystis ulmi* (23). The dsRNAs of *R. solani* AG-1 IA and IB were located in the microsomal fraction of the fungal cell. However, we also observed weak bands from the cell-debris fraction that could be contamination occurring during cell fractionation.

The genetic diversity of dsRNAs from *R. solani* belonging to five anastomosis groups (AGs-1–5) has been reported previously (3). Similarly, we observed extensive variation in the dsRNA profiles of 30 isolates belonging to AG-1 IC, AGs-2–9, and AG-BI. Isolates of AGs-6, -8, and -9 did not contain dsRNA. Although we recorded mycelial growth rate and phenol oxidase activity of the isolates from different anastomosis groups, we did not attempt to compare these characters among the dsRNA-containing and dsRNA-free isolates, because few isolates were tested within each anastomosis group. The dsRNA profiles of the other anastomosis groups were different from those observed for AG-1 IA or IB.

Castanho et al (5–7) reported that hypovirulence in *R. solani* was due to the presence of dsRNA. However, other researchers have reported no consistent association of dsRNA with the degree of pathogenicity of the isolates (3,13,30). Significant correlations between the presence of dsRNA and phenol oxidase activity or virulence was found in the case of *C. parasitica* (22) and *P. infestans*; a correlation between the presence of dsRNA and higher mycelial dry weight was observed (26). The results of our experiments indicate that the presence of dsRNA in *R. solani* does not appear to have any effect on the virulence, mycelial growth, or phenol oxidase activity of the AG-1 IA or IB isolates. However, further research needs to be conducted to determine the actual role of dsRNA in *R. solani* AG-1 IA and IB isolates and how this role relates to Rhizoctonia foliar blight of soybean.

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