Characterization and Pathogenicity of *Rhizoctonia* spp. Isolated from Rice, Soybean, and Other Crops Grown in Rotation with Rice in Texas

R. K. JONES, Extension Plant Pathologist, and S. B. BELMAR, Former Graduate Student, Department of Plant Pathology and Microbiology, Texas A&M University System, College Station 77843

ABSTRACT

Jones, R. K., and Belmar, S. B. 1989. Characterization and pathogenicity of *Rhizoctonia* spp. isolated from rice, soybean, and other crops grown in rotation with rice in Texas. Plant Disease 73:1004-1010.

Isolates of *Rhizoctonia* spp. collected from diseased rice (*Oryzae sativa*) sheaths were characterized as *R. solani* AG-1 IA (sasakii form), *R. oryzae*, and a previously undescribed collection of isolates of *R. solani* designated AG-UNK that failed to anastomose with AG tester isolates 1 through 8 of *R. solani* but anastomosed strongly with each other. *R. solani* AG-1 IA and AG-1 IB (web blight form) were recovered from diseased foliage of soybean (*Glycine max*) showing characteristic signs and symptoms of aerial blight and web blight, respectively. Isolates of AG-1 IA also were identified from foliage of diseased sorghum (*Sorghum bicolor*) and corn (*Zea mays*). The teleomorphs of AG-1 IA, AG-1 IB, and AG-UNK were observed and conformed to the genus *Thanatephorus*. The teleomorph of *R. oryzae* was observed and conformed to descriptions of the genus *Waitea*. A previously undescribed *Ceratobasidium* sp. was recovered frequently from rice sheaths showing no disease symptoms. In the greenhouse, pathogenicity on young foliage of rice and soybean varied among groups, with isolates of *R. solani* AG-1 IA and *R. solani* AG-UNK more virulent on rice and isolates of AG-1 IA, AG-1 IB, and AG-UNK more virulent on soybean. Only isolates of *R. solani* AG-1 IA significantly reduced rice yields in the field, however.

Additional keywords: banded leaf and sheath blight, sheath blight, sheath spot

Rice (*Oryzae sativa* L.) is produced on 100,000–140,000 ha in the Texas upper gulf coast. The most significant disease affecting rice production in Texas and the United States during the last decade is sheath blight caused by *Rhizoctonia solani* Kühn (teleomorph = *Thanatephorus cucumeris* (Frank) Donk) (25).

Soybean (*Glycine max* (L.) Merrill) has been a popular rotational crop for Texas rice producers during the last two decades. However, economic considerations and the contribution of soybean rotations to enhanced damage from the sheath blight fungus in subsequent rice crops (6) is causing a shift in rotational patterns to include corn (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L.) Moench) and to increased fallowing of land to native pastures of bermudagrass (*Cynodon dactylon* (L.) Pers.).

Belmar et al (6) surveyed commercial rice fields in Texas that had various crop rotational patterns involving soybean or bermudagrass pastures. They found that disease incidence in the rice crop could be predicted using a preplant soil assay for sclerotia of *R. solani* AG-1 IA. Sheath blight is initiated in rice by these large buoyant sclerotia that serve as inoculum for infecting rice tillers at the waterline after permanent flood is established. In-season treatment thresholds for chemical control of the disease were developed by Jones et al (23). Both the preplant soil assay and the in-season scouting procedures rely on an accurate determination of signs and symptoms of the causal fungus.

The purpose of this investigation was to characterize the *Rhizoctonia* spp. recovered from blighted rice sheaths, rice soils, and other crop plants, including soybean, sorghum, and corn, grown in rotation with rice in Texas. Characterization includes descriptions of the signs of these fungi and disease symptoms on hosts, the habits of isolates in culture, and the pathogenicity of isolates.

Present address of first author: Department of Plant Pathology, University of Minnesota, St. Paul 55108.

Accepted for publication 26 June 1989 (submitted for electronic processing).

MATERIALS AND METHODS

Isolation of fungi from soil and plant tissue. Isolates of *R. solani* and other *Rhizoctonia* spp. were collected from 68 fields in southeastern Texas during 1984–1987. Rice had been grown at least once in the 4-yr period prior to sampling. Diseased plant tissue was obtained 45–60 days after emergence. Isolates from lesions were obtained by plating tissue on 1.5% water agar amended with streptomycin sulfate and penicillin G, each at 50 mg/L (WA^+). Hyphal tips of isolates were transferred to potato-dextrose agar (PDA). Sclerotia were recovered from soil and also collected directly from the surface of diseased plant material. Collections from soil were made before planting, and sclerotia were removed by elutriation and sieving onto screens with 600- and 150-μm openings (6). Isolates from sclerotia were obtained by germinating sclerotia on WA^+ and transferring hyphal tips to PDA.

Teleomorphs were collected from plant parts 60–120 days after emergence. Collections were also made from ratoon crops of rice and sorghum. Small portions of the hymenia were scraped from the plant tissue and plated on WA^+, and hyphal tips were transferred to PDA to confirm the identity of the anamorph. Hymenia also were obtained directly from plant material by making cellophane tape mounts from specimens in the field during early morning hours (before 0600 hours) and fixing these to a glass microscope slide.

Characterization of the anamorphs. The various field isolates were grown on PDA incubated at 28 C for 14 days and compared for colony morphology and size and shape of sclerotia. On this basis, isolates could be separated into five groups.

The radial growth rate (mm/hr) of six isolates from each of the five groups was measured at 28 and 35 C on PDA after incubation in the dark for 36 hr. A completely randomized design with four replicate plates per isolate was used. Data were analyzed by analysis of variance, and mean separations were performed with Duncan's multiple range test.

The reaction of isolates to phenol was tested on actively growing 5-day-old PDA cultures of six isolates from each of the five groups. A drop of lactophenol was placed directly on the mycelium at four locations equidistant between the center of the colony and the colony edge. Quadruplicate cultures were incubated in the dark for 48 hr at 28 C before reaction was evaluated.

The nuclear condition in penultimate cells of vegetative hyphae was determined by the DAPI stain technique (27). Fifty cells from each of 16 isolates (four in each of four multinucleate isolate groups) were examined. The diameter of the penultimate cell in young vegetative hyphae was measured from these same preparations.

Characterization of the teleomorphs. Teleomorphs produced on rice, soybean, sorghum, and corn plants were examined in vivo on cellophane tape mounts stained with trypan blue/lactophenol. In vitro production of the teleomorph was

attempted for selected isolates by the modified soil-over-agar culture method (53) and the soil-agar flask method (11). The number of nuclei in basidiospores of *R. solani* AG-1 IA was determined by staining cellophane tape mounts of field-collected hymenia directly with a 10-ng/L solution of DAPI in 70% ethanol.

Anastomosis group (AG) determinations. Anastomosis testing was performed by opposing field isolates with tester isolates (Table 1) on glass slides coated with 1.5% water agar (28). Pairings in which anastomosis was not observed with any tester were repeated at least 10 times. A minimum of three imperfect fusions (59) in at least one test was required to consider an unknown isolate to be a member of a particular anastomosis group. Simple contact of hyphae was scored negatively.

Pathogenicity testing. Comparative pathogenicity of the *Rhizoctonia* spp. was evaluated in greenhouse tests using 21-day-old rice (cv. Lemont) and 28-day-old soybean (cv. Ransom) plants as described by Belmar et al (6). Treatments consisted of four isolates from each of the five groups. Treatments included an uninoculated control and were replicated three times. The experiment was repeated twice. Pots containing plants were arranged in a completely random design and incubated 7 days under a plastic tent in the greenhouse. Disease was rated on a 0–5 scale, where 0 = no disease, 1 = 1–20%, 2 = 21–40%, 3 = 41–60%, 4 = 61–80%, and 5 = 81–100% foliar blight of the plant canopy.

The comparative pathogenicity of isolate groups on field-grown rice was evaluated at the L. E. Crane Research Station in Alvin, Texas, in 1986. Field plots (2 × 3 m) were replicated five times in a randomized complete block design. Plots consisted of five drill rows of rice (cv. Lemont) at 15-cm spacings. The middle row was inoculated with 1,000 ml of an aqueous inoculum suspension at 50 days after emergence. Inoculum was prepared by homogenizing 14-day-old cultures grown on rice hull agar (6) and mixing at a rate of one 9-cm-diameter plate per 200 ml of water. One isolate from each of four groups—*R. solani* AG-1 IA, AG-1 IB, and AG-UNK and *R. oryzae*—were tested. Treatments also included an uninoculated control. At harvest, panicles from the middle row were removed by hand and the grain was threshed, dried, and weighed. Data were analyzed by analysis of variance, and mean separations were performed by Duncan's multiple range test.

RESULTS

Isolation of fungi from soils and plant tissue. Of the 304 isolates recovered from rice sheath lesions, teleomorphs, or sclerotia, 227 were *R. solani* AG-1 IA and 44 were *R. oryzae*. An unknown group of 17 multinucleate isolates did not anastomose with any of the AG testers but anastomosed with each other; this group showed all the characteristic features of the genus *Rhizoctonia* (35,42), including brown pigmentation in culture, and will be referred to as *R. solani* AG-UNK. Additionally, 16 isolates of a uninucleate fungus were recovered from the surfaces of rice plants with no apparent disease.

Sheath blight lesions appeared on rice plants in surveyed fields within 5–7 days after flood was established (40–55 days after planting) at or near the paddy surface. Lesions initially were 2 × 1 cm, water-soaked, and devoid of obvious aerial mycelium. Lesions enlarged rapidly, and aerial hyphae were evident within 3 wk of initial infection. Pathogen spread among plants was by contact between diseased and healthy leaves. Hyphae also grew from initial lesions across the paddy surface to contact healthy tillers. Mature sclerotia of the sheath blight fungus were dark brown, 1.5–3.0 mm in diameter, and generally spherical with a slight concave depression on the underside. Sclerotia were produced on diseased plants and were recovered from soil on sieves with 600-μm openings.

Sheath spot lesions were typically oval and 1–3 cm long and 1–2 cm wide, with a uniformly darkened (water-soaked) surface devoid of obvious aerial mycelium when samples were collected in the paddy during the vegetative stage of the rice plant. Sheath spot lesions did not enlarge rapidly under field conditions and typically remained as discrete areas of damage on the outermost leaf sheath just below and opposite the ligule. Sclerotia of *R. oryzae* were not recovered from plants or soil, and this organism was isolated only from rice. Isolates of *R. oryzae* could be recovered by plating plant residue after elutriation.

The isolates designated *R. solani* AG-UNK were recovered from diseased rice sheaths showing symptoms indistinguishable from those caused by *R. oryzae*. Sheath lesions caused by this fungus were observed relatively late in the growing season (70–100 days after planting), whereas lesions of sheath blight and sheath spot could be detected as early as 50 days after planting. Sclerotia of *R. solani* AG-UNK were recovered occasionally from elutriated soil, on sieves with 600-μm openings, as "suspected" sclerotia of *R. solani* AG-1 IA. They were typically 1.5–2.0 mm in diameter and punky in texture. Sclerotia were not observed on host plants, and this fungus was not isolated from any crop other than rice. *R. solani* AG-UNK was recovered from diseased rice sheaths, rice soils, and rice residues in four geographically separated counties in the Texas upper gulf coast but at an extremely low frequency.

Table 1. Isolates of *Rhizoctonia* spp. used as tester strains for anastomosis group determinations

Isolate	Anastomosis group	Host	Source of isolate[z]
R. solani			
NGW	AG-1 IA	Rice sheath, Texas	9
33-6A	AG-1 IA	Rice sheath, Texas	5
R-245	AG-1 IB	Bean leaves, Costa Rica	1
EE-NC	AG-1 IB	Bean leaves, North Carolina	2
R-43	AG-1 IC	Pine seedling, Canada	1
RSR	AG-1 IC	Radish root, Florida	3
Rhs-45	AG-2-1	Peanut root, Georgia	7
Rhs-36	AG-2-2	Corn root, Georgia	7
TR-2	AG-2-2	St. Augustinegrass, Texas	5
R-141	AG-3	Soil, Maryland	1
R-283	AG-4	Conifer seedling, California	1
MAR-1	AG-4	Cotton seedling, Texas	6
Devay-1	AG-4	Cotton seedling, California	6
R-441	AG-5	Soil, Japan	7
NHA2-1	AG-6	Soil, Japan	8
1535	AG-7	Soil, Japan	8
A125	AG-8	Wheat root, Washington	8
Waitea circinata			
G37	WAG	Turfgrass, New Zealand	4
241	WAG	Soil, Australia	4
R. oryzae			
137	WAG-O	Rice sheath, California	4
537	WAG-O	Rice sheath, Arkansas	4
541	WAG-O	Rice sheath, Arkansas	4
R. zeae			
215	WAG-Z	Bentgrass, North Carolina	4
C2	WAG-Z	Corn, North Carolina	4

[z] 1 = E. E. Butler, University of California, Davis; 2 = E. E. Echandi, North Carolina State University, Raleigh; 3 = M. P. Grisham, USDA, Houma, Louisiana; 4 = P. S. Gunnell, University of California, Davis; 5 = R. K. Jones (this study); 6 = R. W. Jones, University of California, Berkeley; 7 = S. B. Martin, Connecticut Agricultural Experiment Station, New Haven; 8 = C. M. Rush, Texas Agricultural Experiment Station, Bushland; and 9 = N. G. Whitney, Texas Agricultural Experiment Station, Beaumont.

The previously undescribed uninucleate fungus was observed on symptomless rice sheaths. Sclerotia of this fungus were not observed on host plants and were not recovered from soil.

Twenty-five isolates recovered from soybean leaves with aerial blight were characterized as *R. solani* AG-1 IA, and 12 isolates from web-blighted soybean leaves were identified as *R. solani* AG-1 IB. Although symptoms of aerial blight and web blight on soybean plants were sometimes difficult to differentiate, the size and shape of sclerotia, when present, were sufficiently characteristic to distinguish between the two causal agents. Sclerotia of *R. solani* AG-1 IA are sometimes larger and more amorphous on soybean (Fig. 1A) than on rice, corn, and sorghum plants. Amorphous sclerotia were frequently recovered from field soils on which soybeans had been grown the previous season.

Isolates designated *R. solani* AG-1 IB were recovered from diseased soybean foliage but not from soil. Sclerotia on affected plants were small (0.05–0.25 mm in diameter) and very numerous on leaves and petioles. Sclerotia collected from plants easily passed through the openings of 150-μm sieves.

Twenty-six isolates of *R. solani* AG-1 IA were recovered from the foliage of diseased corn or sorghum grown in rotation with rice. Plants showed symptoms typical of banded leaf and sheath blight (3,32,36).

Characterization of the anamorphs. The appearance of isolates of *R. solani* AG-1 IA on PDA fit the description of *R. solani* AG-1 type 2 given by Sherwood (46) and that of *Pellicularia filimentosa* f. sp. *sasakii* given by Exner (12). Colonies of *R. solani* AG-1 IB on PDA matched the description of *R. solani* AG-1 type 1 of Sherwood (46) and of *P. f.* f. sp. *microsclerotia* of Exner (12). Both groups could be easily distinguished from tester isolates of AG-1 IC (Table 1), which resemble the description of *R. solani* AG-1 type 3 given by Sherwood (46).

Colonies of *R. oryzae* on PDA were white initially but turned pinkish within 14 days. Amorphous, pink to salmon sclerotia-like bodies similar to those described by Ryker and Gooch (44) were produced submerged in culture. Five of 114 isolates examined, however, produced distinctly round, firm, red sclerotia (0.5–1.0 mm in diameter) submerged in the culture medium, along with the amorphous pink type, after 14 days of incubation. The production of round, red sclerotia is characteristic of isolates of *R. zeae* Voorhees (55). Repeated subculturing of hyphal tips produced by germinating these sclerotia on WA^+ failed to modify the production of two distinct sclerotial types in these isolates.

In PDA cultures, the AG-UNK isolates of *R. solani* were initially white but turned brown within 7–8 days. Prominent sclerotia were absent, but small (0.2–0.3 mm in diameter) sclerotial initials formed frequently in the aerial hyphae of 7- to 10-day-old cultures. These initials never developed into distinct sclerotia. In gross morphology and coloration, isolates of this group most closely resembled isolates of *R. solani* AG-2-2.

Isolates of the uninucleate fungus recovered from symptomless rice sheaths produced white colonies on PDA that turned buff-colored after 14 days. Sclerotia were not formed in culture. This fungus possessed dolipore septa as well as other features characteristic of the genus *Rhizoctonia* (35). Isolates of this fungus will be hereafter referred to as *Rhizoctonia* sp. Other *Rhizoctonia*-like fungi have been described that possess dolipore septa and uninucleate hyphae (7,21).

Isolates of the five *Rhizoctonia* spp. differed significantly in growth rate on PDA (Table 2). Isolates of *R. solani* AG-1 IA grew fastest at 28 C but slowest at 35 C. Growth rates of isolates of *R. solani* AG-1 IB also were greatly reduced at the higher temperature. Growth rates of isolates of *R. oryzae* were not significantly different ($P = 0.05$) from isolates of *R. solani* AG-1 IA at 28 C but were significantly faster than all groups at 35 C. Isolates of *R. solani* AG-UNK and of the uninucleate *Rhizoctonia* sp. were intermediate in growth rate, and growth appeared less affected by temperature (Table 2).

Isolates of *R. oryzae* turned dark brown in response to phenol, but all other isolate groups reacted negatively to this compound (Table 2). These results are similar to those observed by Martin and Lucas (28).

Penultimate cells of the four multinucleate groups contained 4–23 nuclei, with an average of 9.6. There were no significant differences ($P = 0.05$) in the number of nuclei per cell between isolate groups (Table 3) or between isolates within groups. Nuclear division appeared to be conjugate (13). Numbers of nuclei per cell in all groups of isolates were distributed normally about the mean.

The penultimate cells of *R. oryzae* isolates were significantly ($P = 0.05$) narrower than those of the other multinucleate groups studied (Table 3). When measured in these preparations, the penultimate cells of *R. solani* isolates were slightly smaller than the 6–10μ reported for 3-day-old hyphae of the species by Sherwood (46).

Characterization of the teleomorphs. Hymenia of *R. solani* AG-1 IA were observed on rice, soybean, sorghum, and corn. Fructifications on rice occurred on the outermost leaf sheath and extended 20–30 cm up from the waterline. Fertile hymenia also formed on the abaxial leaf surface after extended periods of high humidity. Fructifications formed over apparently undamaged tissue (Fig. 1B), and fertile areas along these hymenia were discontinuous. Hymenia were white in the early morning hours and turned buff-colored as the relative humidity decreased later in the day. Mature basidia-bearing sterigma and basidiospores were most easily observed on plants collected before 1000 hours. Although fertile hymenia of *R. solani* AG-1 IA were not produced in vitro with the modified soil-over-agar (53) or soil-agar flask (11) techniques, telemorphs of

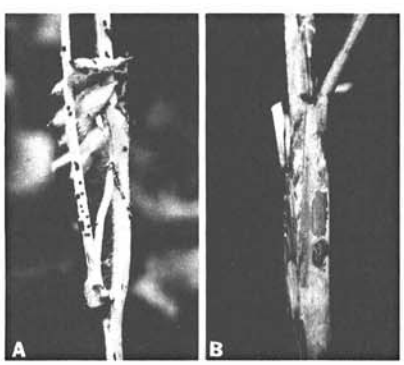

Fig. 1. Signs and symptoms of *Rhizoctonia solani* AG-1 IA. **(A)** Mature sclerotia of the fungus on soybean plant affected with aerial blight. **(B)** Hymenia of *Thanatephorus cucumeris*, anamorph *R. solani* AG-1 IA, on rice sheath; portion of hymenium removed by cellophane tape overlies healthy tissue.

Table 2. Radial growth rate and phenol reaction of isolates of *Rhizoctonia solani*, *R. oryzae*, and a uninucleate *Rhizoctonia* sp. isolated from rice and soybean

Isolate group	Growth rate (mm/hr)[y]		Reaction to phenol[z]
	28 C	35 C	
R. solani AG-1 IA	0.80 a	0.23 c	−
R. solani AG-1 IB	0.59 bc	0.05 d	−
R. solani AG-UNK	0.53 bc	0.42 b	−
R. oryzae	0.66 ab	0.82 a	+
Rhizoctonia sp.	0.47 c	0.37 b	−

[y] Radial growth rate as determined after 36 hr of incubation in the dark on PDA. Values are means of six isolates within each group. Values within columns followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

[z] Reaction of 5-day-old cultures to lactophenol after 48 hr at 28 C. Six isolates within each group were tested. + = Development of a brown pigment in the presence of lactophenol.

R. solani AG-1 IA collected from field specimens conformed to descriptions of *T. cucumeris* (50,51).

Examination of the nuclear condition in 908 basidiospores (227 basidia) from 14 hymenia of *R. solani* AG-1 IA collected from rice sheaths revealed that 88.3% were uninucleate, 5.8% were binucleate, 0.6% were trinucleate, and 5.3% were without nuclei. Binucleate and trinucleate spores were usually accompanied by an appropriate number of basidiospores without nuclei, suggesting imperfect migration of nuclei from the metabasidium (13) and the potential for a heterokaryotic condition in cultures derived from such spores. Fifteen of the 53 binucleate spores (28.3%) were in spore tetrads without corresponding anucleate spores, suggesting that the binucleate condition in these spores may have resulted from mitotic division before spore detachment.

The teleomorph of *R. oryzae* was observed frequently on rice during periods of high relative humidity after the crop entered the heading growth stage. Hymenia were pink, resupinate, and pruniose, becoming waxy during periods of low relative humidity. The hymenia generally extended 3–5 cm over the surface of typical sheath spot lesions and overlay diseased and apparently healthy tissue. Basidia were not cylindrical but often were constricted about the middle, giving them a suburniform shape. Hymenia branches were involute, occasionally approaching circinate. Basidia and basidiospore dimensions and arrangement were as described by Oniki et al (40).

Fertile hymenia of *R. oryzae* could be produced in vitro with certain isolates. Secondary septation of sterigmata and basidiospores (up to two per spore) was observed as these cultures aged. Septation in the metabasidium or repetitive spore germination was not observed. The perfect stage of *R. oryzae* from rice in Texas conformed in general with the genus *Waitea* (52) and in detail with *W. circinata* described on rice in California affected with sheath spot (20).

The teleomorph of the *R. solani* designated AG-UNK was observed on rice sheaths unaffected by any of the sheath-spotting fungi and also was recovered from rice sheaths showing lesions similar to those from which *R. oryzae* was recovered. Fructifications of the unknown isolates of *R. solani* corresponded to descriptions of *T. cucumeris* (50,51). Hymenia were much less dense than those of the sheath blight fungus. They could be detected in the early morning when dew was on the plant surface but were much less evident later in the day. Single basidiospore isolates anastomosed readily with anamorphs designated *R. solani* AG-UNK but not with any tester isolate. In vitro techniques to produce the teleomorphs of isolates in this group were unsuccessful. Studies to further characterize this group are under way.

The teleomorph of the uninucleate *Rhizoctonia* sp. was observed on rice sheaths unaffected by disease. Fructifications formed on apparently healthy tissue extending 6–8 cm up the sheath from the waterline to the juncture of the leaf blade. Hymenia were white and effuse and occurred in small inconspicuous patches. Basidia were sphaero- to pyropedunculate, 9.9 × 11.3 μm, and produced in a raceme-like arrangement. Basidia typically were 2.0–2.5 times as wide as the supporting hyphae and produced four stout, cornute sterigmata of variable length up to 4.4 μm. Basidiospores were hyaline and globose (6.8 × 5.9 μm) with a pointed apiculus. Single basidiospore isolates of this fungus were uninucleate. The perfect state of this fungus conformed to the genus *Ceratobasidium* (50,51). Repetitive germination of basidiospores was observed. In vitro production of the teleomorph was not attempted.

Teleomorphs of *R. solani* AG-1 IB were observed on soybean leaves affected by web blight. Buff-colored hymenia were produced on the undersides of leaves 1–2 cm in advance of necrotic lesions. The general morphology of the hymenia was examined and, although basidiospores were few, conformed to that of *T. cucumeris* (50,51) and also to descriptions of *Corticium microsclerotia* (56,57) and *P. f.* f. sp. *microsclerotia* (12). The perfect stage of the web blight fungus has not been described previously in studies that included anastomosis determinations. Repeated attempts to produce the perfect state of this fungus in vitro were unsuccessful.

Anastomosis group determinations. Isolates designated *R. solani* AG-1 IA or *R. solani* AG-1 IB anastomosed with all six tester isolates designated *R. solani* AG-1 (Table 1). The frequency of anastomosis was highest when pairings were made between isolates within the same intraspecific group or ISG (34,35). For example, AG-1 IA × AG-1 IA or AG-1 IB × AG-1 IB pairings anastomosed more frequently than pairings made between isolates of AG-1 IA × AG-1 IB.

All 17 isolates of *R. solani* AG-UNK anastomosed with one another but not with tester isolates of any other AG group of *R. solani*. Death of anastomosed cells in certain isolate pairings within this group was evident 6 hr after hyphal contact.

Isolates of *R. oryzae* anastomosed with three tester isolates designated *R. oryzae* that were originally recovered from rice sheath spots in Arkansas and California (Table 1). All isolates designated *R. oryzae*, including those with the embedded round, red sclerotia, failed to anastomose with the two tester isolates designated *R. zeae*. These findings support the conclusions of Oniki et al (40) that these anamorphs can be separated into two groups, WAG-O and WAG-Z, on the basis of anastomosis determinations.

In 11 pairings, selected isolates of *R. oryzae* anastomosed with tester isolates designated *W. circinata* (Table 1). The frequency of anastomosis was very low, and no pairing ever resulted in three imperfect fusions. Typically, hyphae overlapped with no evidence of interaction.

Pathogenicity. The average disease index of six isolates of *R. solani* AG-1 IA in greenhouse trials was 4.7 on rice and 4.3 on soybean (Table 4). When 72

Table 3. Number of nuclei per cell and hyphal diameter of penultimate cells in vegetative hyphae of isolates of *Rhizoctonia solani* and *R. oryzae*

Isolate group	Number of nuclei per cell	Hyphal diameter (μm)[z]
R. solani AG-1 IA	10.1 a	5.3 a
R. solani AG-1 IB	10.2 a	6.1 a
R. solani AG-UNK	8.3 a	5.1 a
R. oryzae	9.8 a	3.2 b

[z]Isolate groups represent the means of four isolates. Means followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 4. Disease index on rice and soybean in the greenhouse and yields in a field test of rice inoculated with selected isolates of *Rhizoctonia solani*, *R. oryzae*, and a uninucleate *Rhizoctonia* sp.

Isolate group	Greenhouse disease index[x]		Rice field trial	
	Rice	Soybean	Yield (kg/ha)[y]	Loss (%)[z]
R. solani AG-1 IA	4.7	4.3	6,761 b	10.2
R. solani AG-1 IB	1.1	3.7	7,440 a	1.1
R. solani AG-UNK	3.4	3.4	7,458 a	1.0
R. oryzae	0.1	0.2	7,516 a	0.1
Rhizoctonia sp.	0.0	0.0	NT	NT
Uninoculated	0.0	0.0	7,526 a	...

[x]Foliar damage on a scale of 1–5, where 1 = 10–20% damage and 5 = 81–100% damage. Values are means of four isolates per group replicated three times and averaged over three trials.

[y]Based on five replicates of a 0.53-m^2 harvested area. Values followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test. NT = not tested.

[z]Calculated relative to the yield in uninoculated plots. NT = not tested.

additional isolates, representing collections from 24 fields, were screened for virulence on rice (S. B. Belmar, *unpublished*), no significant ($P = 0.05$) differences were observed, regardless of whether the isolates came from severely affected fields or fields with little inoculum or disease. Avirulent isolates were not identified. An isolate of *R. solani* AG-1 IA was the only *Rhizoctonia* spp. to significantly ($P = 0.05$) reduce rice yields in the field (Table 4).

Web blight isolates (AG-1 IB) were moderately virulent on rice but more virulent on soybean foliage in the greenhouse (Table 4). Significant ($P = 0.05$) reductions of yields were not observed in the field trial of rice inoculated with an isolate of this group. Similarly, isolates of *R. solani* AG-UNK were virulent on rice and soybean foliage in the greenhouse, but yield loss was not significant in the field (Table 4).

Six isolates of *R. oryzae* were weakly virulent on rice and soybean foliage in greenhouse tests. Rice leaves contacting the inoculum plug became diseased, but leaf-to-leaf spread of the fungus resulting in a "foliar blight" was not observed. One isolate of this group was tested in the field and did not significantly ($P = 0.05$) reduce rice yields (Table 4).

Isolates of the uninucleate *Rhizoctonia* sp. did not cause symptoms on rice or soybean foliage in the greenhouse (Table 4). Isolates of this group were not tested in the field.

DISCUSSION

The aquatic environment involved in paddy rice production supports diverse populations of *Rhizoctonia* spp. The frequency of isolation of particular components of this community varies from field to field and can be influenced by production practices. Understanding the relative yield loss contribution of each component is important to accurate utilization of preplant (6) or in-season (23) thresholds designed to minimize loss. We found that only *R. solani* AG-1 IA, the causal agent of sheath blight, was present in significant numbers and resulted in sufficient damage to warrant expensive chemical control measures (23) for rice grown in Texas. Other *Rhizoctonia* spp. were present in paddies but caused limited damage to rice because of low virulence or low incidence.

In the 1930s, Ryker (43) and Ryker and Gooch (44) characterized the *Rhizoctonia* spp. isolated from rice sheaths in Louisiana. *R. oryzae* was the predominant species recovered, with *R. zeae* and *R. solani* (sasaki form) identified only rarely. By the mid 1970s, 60% of surveyed fields in southwestern Louisiana were found to contain an average of 7% of the tillers infected with the sheath blight fungus (45).

Marshall and Rush (26) showed that the initial infection parameters of isolates of *R. solani* AG-1 IA and *R. oryzae* are identical. However, we have shown that plant-to-plant spread and subsequent yield loss caused by isolates of the two fungi differ considerably. Sheath spot lesions remain limited to the outer rice sheaths (44), and the fungus does not invade the culm (26). Sheath blight lesions spread rapidly up the plant and to other plants by aerial growth of vegetative hyphae. Spread of *R. solani* AG-1 IA by plant-to-plant contact is enhanced in intensive production systems that utilize susceptible semidwarf cultivars and high-nitrogen fertilizer programs (6,23).

Isolates of *R. solani* AG-1 IA attack a large number of crop plants and weeds (49). In most cases, the disease is called aerial blight. Exceptions include sheath blight of rice and banded leaf and sheath blight of sorghum (32,36) and corn (3). Shurtleff (47) identified the cause of banded leaf and sheath spot of corn as *R. microsclerotia* Matz, although his illustrations clearly show characteristic sasakii-form sclerotia. O'Neill and Rush (36) described the anamorph and teleomorph of the sheath blight fungus on sorghum in Louisiana and demonstrated that isolates from sorghum, rice, and bermudagrass were *R. solani* AG-1. During our study, we observed *R. solani* AG-1 IA causing typical banded leaf and sheath blighting symptoms on sorghum and corn. In both cases, teleomorphs conforming to *T. cucumeris* could be collected from abaxial leaf surfaces, and cultures made from such isolations produced sclerotia characteristic of AG-1 IA that would anastomose with AG-1 tester isolates.

Rhizoctonia diseases affecting the foliage of soybean include Rhizoctonia aerial blight (49). Atkins and Lewis (5) reported "Rhizoctonia aerial blight" of soybean in Louisiana during the 1950s and described the causal agent as *R. microsclerotia* (syn. *C. microsclerotia* (Matz) Weber); disease signs, however, included production of small (0.10- to 0.17-mm) sclerotia on the host. O'Neill et al (37) reported that aerial blight of soybean is caused by *R. solani* AG-1. Her description of disease signs included the presence of characteristically large (1- to 6-mm) sclerotia, and she concluded that aerial blight was caused by the "sasakii type" of *R. solani* and not by the "microsclerotial type" reported earlier by Atkins and Lewis (5).

Matz first described *R. microsclerotia* from fig (*Ficus carica* L.) in Florida in 1917 (29). Weber (56,57) reported extensive damage to bush snapbean (*Phaseolus vulgaris* L.) fields in Florida caused by a fungus equated with *R. microsclerotia* but, observing the perfect stage on the undersurfaces of web blighted leaves, named the causal agent for the teleomorph as *C. microsclerotia*.

Exner (12) included isolates from Weber in her comparative study of four *Rhizoctonia* spp. occurring in Louisiana. She concluded that the fungus causing web blight of various hosts, including fig, snap bean, and lima bean (*P. lunatus* L.), in Louisiana was the same as that described by Weber (56). She named the causal agent *P. filamentosa* (Pat.) Rogers f. sp. *microsclerotia* (Matz) Exner.

Rhizoctonia web blight affects dry bean (9,11,16,17), snap bean (12,56), cowpea (*Vigna unguiculata* (L.) Walp.) (38), and soybean (9,22) in many tropical and subtropical areas of the world. Certain isolates of the web blight fungus from Costa Rica (11) were found to belong to AG-1 by Parmeter et al (42). Galindo et al (15) also identified web blight isolates from Colombia, South America, as AG-1.

Soybeans in Texas are affected by both Rhizoctonia aerial blight (*R. solani* AG-1 IA) and Rhizoctonia web blight (*R. solani* AG-1 IB). Differentiation between these intraspecific groups (ISGs) of AG-1 is critical to understanding inoculum density/disease incidence relationships in either rice or soybean and is particularly important in intercropping (16) or rotational cropping (6) decisions. Temperature optima, fungicide sensitivity, and conditions favoring propagule survival can and do vary between these two ISGs. Resistance screening programs also will be enhanced by the appropriate selection of isolates.

Anastomosis group 1 of *R. solani* consists of the intraspecific groups AG-1 IA, AG-1 IB, and AG-1 IC (34,35), corresponding to "cultural types" 2, 1, and 3, respectively, of Sherwood (46). AG-1 IA is the sasakii form of *R. solani* AG-1 and causes sheath blight of rice, aerial blight of soybean, and banded leaf and sheath blight of sorghum and corn in Texas. The perfect stage of this fungus possesses morphological features within the range of *T. cucumeris* (50,51). Attempts at in vitro production of the teleomorph with isolates of AG-1 IA were unsuccessful in this study as well as in many others (2,10,33,36). Oniki et al (39) recently described a soil-over agar technique in which humidity was regulated that resulted in the successful fructification (fruiting) of six Japanese isolates of the sheath blight fungus.

AG-1 IB is the web blight form of *R. solani* AG-1. The perfect stage of this fungus shows morphological features within the range of *T. cucumeris* (50,51). Repeated attempts to induce the teleomorph stage of web blight isolates by the soil-over-agar method or the soil-agar flask method were unsuccessful. Failure to produce the teleomorph of AG-1 IB isolates in vitro using the soil-over-agar method (14,33,38) or the nutrient stepdown technique (2,14) has been reported.

Echandi (11) made over 300 isolations from dry bean leaves with web blight in

Costa Rica. Some of these isolates were induced to fruit by the soil-agar flask method he described. Parmeter et al (42) conducted anastomosis tests on Echandi's isolates of *P. f.* f. sp. *microsclerotia* and found that some belonged to AG-1 and some to AG-2. Stretton and Flentje (48) studied the AG-2 isolates of Echandi and reported that they would not cause a foliar blight of bean leaves but were pathogenic to cowpea stems and fruited in vitro.

AG-1 IC represents the third ISG of *R. solani* AG-1. It appears to be only mildly virulent and to be prevalent under cooler climatic conditions. It has been reported from numerous hosts, including pine and soybean seedlings in Canada (42), sugar beet and buckwheat in Japan (35), flax (4,18) and carrot (19) in Minnesota, and lima bean (10) in New York. In a study we performed, the index of foliar blighting for two AG-1 IC isolates averaged only 0.5 (1–5 scale) on rice and soybean leaves (R. K. Jones, *unpublished*).

The teleomorph of AG-1 IC is readily produced in culture (2,10,18,39,58). Although isolates of this fungus have been referred to as microsclerotial types (10,19) or as the microsclerotial form (4) of AG-1, they belong to a separate ISG from fungi causing web blights that have been identified as *R. microsclerotia* (9), *C. microsclerotia* (11,56,57), or *P. f.* f. sp. *microsclerotia* (12). These fungi cause economically important web blights of various hosts in tropical and subtropical areas of the world and belong to AG-1 IB of *R. solani.*

The binomial *R. microsclerotia* has also been used to identify fungi belonging to diverse taxa. Mundkur (31) used the name to describe *Sclerotium hydrophilum* Sacc., a weak pathogen of rice, from India. Isolates were verified by Matz and deposited by Mundkur in the Imperial Mycological Institute (IMI 38957). This misidentification was discovered by Mordue (30). *S. hydrophilum* was recovered in our study both as sclerotia on the nodes and ligules of maturing rice plants and from elutriated soil on sieves with 150-μm openings. Superficially, isolates of *S. hydrophilum* resemble members of AG-1 IC of *R. solani* in morphology and colony coloration on PDA. Dolipore septa are present and hyphae are binucleate. Isolates of *S. hydrophilum*, however, possess sclerotia that have a differentiated black rind (at maturity) and a pure white medulla. Park and Bertus (41) called *S. hydrophilum* from rice in Ceylon the Rhizoctonia B strain.

Fourteen ISGs of *R. solani* are currently recognized (8,35), although additional minor ones undoubtedly will be characterized in the future. Certain anastomosis groups, such as AG-1 and AG-2, contain more than one ISG. Isolates within the same AG but in different ISGs can anastomose by imperfect fusion (59). Whether imperfect fusion between ISGs of the same AG is an important measure of relatedness remains uncertain. DNA base-sequence homologies were less than 70% between isolates of AG-1 IA and AG-1 IB (24) and less than 50% between AG-1 IA and AG-1 IC (54). Intraspecific groups may represent noninterbreeding populations. Hyphal fusion and nuclear exchange have been demonstrated only within ISGs, either as self-anastomosis (perfect fusion) or among paired single basidiospore isolates (1,18,58). Recognition of the ISG as the basic etiological unit will be critical to developing effective control strategies for diseases caused by *R. solani.*

ACKNOWLEDGMENTS

We thank Carol Drees for technical assistance. This project was supported by the Texas Rice Research Foundation.

LITERATURE CITED

1. Adams, G. C., Jr., and Butler, E. E. 1982. A re-interpretation of the sexuality of *Thanatephorus cucumeris* anastomosis group four. Mycologia 74:793-800.
2. Adams, G. C., Jr., and Butler, E. E. 1983. Influence of nutrition on the formation of basidia and basidiospores in *Thanatephorus cucumeris*. Phytopathology 73:147-151.
3. Ahuja, S. C., and Payak, M. M. 1982. Symptoms and signs of banded leaf and sheath blight of maize. Phytoparasitica 10:41-49.
4. Anderson, N. A. 1982. The genetics and pathology of *Rhizoctonia solani*. Annu. Rev. Phytopathol. 20:329-347.
5. Atkins, J. G., Jr., and Lewis, W. D. 1954. Rhizoctonia aerial blight of soybeans in Louisiana. Phytopathology 44:215-218.
6. Belmar, S. B., Jones, R. K., and Starr, J. L. 1987. Influence of crop rotation on inoculum density of *Rhizoctonia solani* and sheath blight incidence in rice. Phytopathology 77:1138-1143.
7. Burpee, L. L., Sanders, P. L., Cole, H., Jr., and Sherwood, R. T. 1980. Anastomosis groups among isolates of *Ceratobasidium cornigerum* and related fungi. Mycologia 72:689-701.
8. Carling, D. E., Leiner, R. H., and Kebler, K. M. 1987. Characterization of a new anastomosis group (AG-9) of *Rhizoctonia solani*. Phytopathology 77:1609-1612.
9. Crispin, A., and Gallegos, C. C. 1963. Web blight—a severe disease of beans and soybeans in Mexico. Plant Dis. Rep. 47:1010-1011.
10. Dillard, H. R. 1987. Characterization of isolates of *Rhizoctonia solani* from lima beans grown in New York State. Phytopathology 77:748-751.
11. Echandi, E. 1965. Basidiospore infection by *Pellicularia filamentosa (Corticium microsclerotia)*, the incitant of web blight of common bean. Phytopathology 55:698-699.
12. Exner, B. 1953. Comparative studies of four Rhizoctonias occurring in Louisiana. Mycologia 45:698-719.
13. Flentje, N. T., Stretton, H. M., and Hawn, E. J. 1963. Nuclear distribution and behavior throughout the life cycles of *Thanatephorus, Waitea*, and *Ceratobasidium* species. Aust. J. Biol. Sci. 16:450-467.
14. Frisina, T. A., and Benson, D. M. 1987. Characterization and pathogenicity of binucleate *Rhizoctonia* spp. from azaleas and other woody ornamental plants with web blight. Plant Dis. 71:977-981.
15. Galindo, J. J., Abawi, G. S., and Thurston, H. D. 1982. Variability among isolates of *Rhizoctonia solani* associated with snap bean hypocotyls and soils in New York. Plant Dis. 66:390-394.
16. Galindo, J. J., Abawi, G. S., Thurston, H. D., and Galvez, G. 1983. Effect of mulching on web blight of beans in Costa Rica. Phytopathology 73:610-615.
17. Galindo, J. J., Abawi, G. S., Thurston, H. D., and Galvez, G. 1983. Source of inoculum and development of bean web blight in Costa Rica. Plant Dis. 67:1016-1021.
18. Garza-Chapa, R., and Anderson, N. A. 1966. Behavior of single-basidiospore isolates and heterokaryons of *Rhizoctonia solani* from flax. Phytopathology 56:1260-1268.
19. Grisham, M. P., and Anderson, N. A. 1983. Pathogenicity and host specificity of *Rhizoctonia solani* isolated from carrot roots. Phytopathology 73:1564-1569.
20. Gunnell, P. S., and Webster, R. K. 1984. The teleomorph of *Rhizoctonia oryzae*. (Abstr.) Phytopathology 74:857.
21. Hall, G. 1986. A species of *Rhizoctonia* with uninucleate hyphae isolated from roots of winter wheat. Trans. Br. Mycol. Soc. 87:466-471.
22. Hepperly, P. R., Mignucci, J. S., Sinclair, J. B., Smith, R. S., and Judy, W. H. 1982. Rhizoctonia web blight of soybeans in Puerto Rico. Plant Dis. 66:256-257.
23. Jones, R. K., Belmar, S. B., and Jeger, M. J. 1986. Evaluation of benomyl and propiconazole for controlling sheath blight of rice caused by *Rhizoctonia solani*. Plant Dis. 70:222-225.
24. Kuninaga, S., and Yokosawa, R. 1982. DNA base sequence homology in *Rhizoctonia solani* Kühn I. Genetic relatedness within anastomosis group 1. Ann. Phytopathol. Soc. Jpn. 48:659-667.
25. Lee, F. N., and Rush, M. C. 1983. Rice sheath blight: A major rice disease. Plant Dis. 67:829-832.
26. Marshall, D. S., and Rush, M. C. 1980. Relation between infection by *Rhizoctonia solani* and *R. oryzae* and disease severity in rice. Phytopathology 70:941-946.
27. Martin, B. 1987. Rapid tentative identification of *Rhizoctonia* spp. associated with diseased turfgrasses. Plant Dis. 71:47-49.
28. Martin, S. B., and Lucas, L. T. 1984. Characterization and pathogenicity of *Rhizoctonia* spp. and binucleate *Rhizoctonia*-like fungi from turfgrasses in North Carolina. Phytopathology 74:170-175.
29. Matz, J. 1917. A *Rhizoctonia* of the fig. Phytopathology 7:110-117.
30. Mordue, J. E. M. 1983. Dolipore septa in *Sclerotium hydrophilum*. Trans. Br. Mycol. Soc. 81:654-655.
31. Mundkur, B. B. 1935. Parasitism of *Sclerotium oryzae* Catt. Indian J. Agric. Sci. 5:393-414.
32. Odvody, G. N. 1986. Banded leaf and sheath blight. Pages 34-35 in: Compendium of Sorghum Diseases. R. A. Frederiksen, ed. American Phytopathological Society, St. Paul, MN.
33. Ogoshi, A. 1976. Studies on the grouping of *Rhizoctonia solani* Kühn with hyphal anastomosis and on the perfect stages of groups. Bull. Natl. Inst. Agric. Sci. Ser. C. 30:1-63.
34. Ogoshi, A. 1985. Anastomosis and intraspecific groups of *Rhizoctonia solani* and binucleate *Rhizoctonia*. Fitopatol. Bras. 10:371-390.
35. Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. Annu. Rev. Phytopathol. 25:125-143.
36. O'Neill, N. R., and Rush, M. C. 1982. Etiology of sorghum sheath blight and pathogen virulence on rice. Plant Dis. 66:1115-1118.
37. O'Neill, N. R., Rush, M. C., Horn, N. L., and Carver, R. B. 1977. Aerial blight of soybeans caused by *Rhizoctonia solani*. Plant Dis. Rep. 61:713-717.
38. Onesirosan, P. T. 1975. Seedborne and weedborne inoculum in web blight of cowpea. Plant Dis. Rep. 59:338-339.
39. Oniki, M., Ogoshi, A., and Araki, T. 1986. Development of the perfect state of *Rhizoctonia solani* Kühn AG-1. Ann. Phytopathol. Soc. Jpn. 52:169-174.
40. Oniki, M., Ogoshi, A., Araki, T., Sakai, R., and Tanaka, S. 1985. The perfect state of *Rhizoctonia oryzae* and *R. zeae* and the anastomosis groups of *Waitea circinata*. Trans. Mycol. Soc. Jpn. 26:189-198.
41. Park, M., and Bertus, L. S. 1934. Sclerotial

diseases of rice in Ceylon. 5. *Rhizoctonia solani* B strain. Ceylon J. Sci. 12:25-36.
42. Parmeter, J. R., Jr., Sherwood, R. T., and Platt, W. D. 1969. Anastomosis grouping among isolates of *Thanatephorus cucumeris*. Phytopathology 59:1270-1278.
43. Ryker, T. C. 1939. The Rhizoctonia disease of Burmuda grass, sugarcane, rice and other grasses in Louisiana. Congr. Int. Soc. Sugar Cane Technol. Proc. 6:198-201.
44. Ryker, T. C., and Gooch, F. S. 1938. Rhizoctonia sheath spot of rice. Phytopathology 28:233-246.
45. Shahjahan, A. K. M., and Rush, M. C. 1979. Occurrence and distribution of diseases caused by sclerotial fungi on rice in Louisiana. Plant Dis. Rep. 63:220-223.
46. Sherwood, R. T. 1969. Morphology and physiology in four anastomosis groups of *Thanatephorus cucumeris*. Phytopathology 59:1924-1929.
47. Shurtleff, M. C. 1980. Compendium of Corn Diseases. 2nd ed. American Phytopathological Society, St. Paul, MN. 105 pp.
48. Stretton, H. M., and Flentje, N. T. 1972. Interisolate heterokaryosis in *Thanatephorus cucumeris* II. Between isolates of different pathogenicity. Aust. J. Biol. Sci. 25:305-318.
49. Stroube, W. H. 1954. Host range of the Rhizoctonia aerial blight fungus in Louisiana. Plant Dis. Rep. 38:789-790.
50. Talbot, P. H. B. 1970. Taxonomy and nomenclature of the perfect state. Pages 20-31 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, ed. University of California Press, Berkeley.
51. Talbot, P. H. B. 1973. Holobasidiomycetidae: Tulasnellales. Pages 317-325 in: The Fungi. Vol. 4A. G. C. Ainsworth, F. K. Sparrow, and A. E. Sussman, eds. Academic Press, New York.
52. Talbot, P. H. B. 1973. Aphyllophorales I: General characteristics; Thelephroid and Cupuloid families. Pages 327-341 in: The Fungi. Vol. 4A. G. C. Ainsworth, F. K. Sparrow, and A. E. Sussman, eds. Academic Press, New York.
53. Tu, C. C., and Kimbrough, J. W. 1975. A modified soil-over-culture method for inducing basidia in *Thanatephorus cucumeris*. Phytopathology 65:730-731.
54. Vilgalys, R. 1988. Genetic relatedness among anastomosis groups in *Rhizoctonia* as measured by DNA/DNA hybridization. Phytopathology 78:698-702.
55. Voorhees, R. K. 1934. Sclerotial rot of corn caused by *Rhizoctonia zeae*, n. sp. Phytopathology 24:1290-1303.
56. Weber, G. F. 1939. Web-blight, a disease of beans caused by *Corticium microsclerotia*. Phytopathology 29:559-575.
57. Weber, G. F. 1951. *Corticium microsclerotia* nom. nov. Mycologia 43:727-728.
58. Whitney, H. S., and Parmeter, J. R. 1963. Synthesis and heterokaryons in *Rhizoctonia solani* Kühn. Can. J. Bot. 41:879-886.
59. Yokoyama, K., and Ogoshi, A. 1986. Studies on hyphal anastomosis of *Rhizoctonia solani* IV. Observation of imperfect fusion by light and electron microscopy. Trans. Mycol. Soc. Jpn. 27:399-413.