

Etiology of Sorghum Sheath Blight and Pathogen Virulence on Rice

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ABSTRACT

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A leaf and sheath blight of sorghum (*Sorghum bicolor*) occurring in 1975, 1976, and 1977 in Louisiana was caused by *Rhizoctonia solani*. This pathogen, which belongs to anastomosis group I, is identical to the fungus that causes aerial blight of soybean (*Glycine max*) and sheath blight of rice (*Oryza sativa*). The perfect state of the pathogen, *Thanatephorus cucumeris*, was found on the foliage of sorghum in several fields. Homokaryotic, single-basidiospore isolates from sorghum varied in cultural characteristics and in virulence to rice cultivars. Virulence of these isolates on sorghum and rice was generally low when compared with heterokaryotic isolates found in the field. Rice cultivars were ranked similarly in disease reaction when inoculated with single-basidiospore isolates or heterokaryotic isolates from sorghum.

Rhizoctonia solani Kühn has been reported as a cause of seedling, crown, sheath, and stem blights and root rots of sorghum (*Sorghum bicolor* (L.) Moench) in several countries (4,10-15). Local epiphytotic on mature grain sorghum foliage have been reported in Georgia and Florida. The pathogens were identified to be *R. solani* (1,17). In 1975, 1976, and 1977 in Louisiana, we observed several sorghum fields with severe blighting of sheaths, leaf blades, and stems. Rainfall during these growing seasons had been unusually heavy. There were also epiphytotic of sheath blight and aerial blight in rice (*Oryza sativa* L.) and in soybean (*Glycine max* (L.) Merrill), also caused by a foliar blighting isolate of *R. solani* (6,7). Sheath blight of rice is considered to be one of the major disease problems of rice in the southern United States (12). Aerial blight of soybeans is endemic in Louisiana, but it caused significant damage in 1973 and 1975 (7).

The causal organism of these diseases is *R. solani*, the anamorphic state of *Thanatephorus cucumeris* (Frank) Donk (13). The perfect state of an aerial blighting form of *R. solani* has infrequently been observed in nature and has not been reported on soybean, rice, or sorghum in the United States.

The present study was initiated to

determine the etiology of sheath blight of sorghum; to compare the *R. solani* strains pathogenic to sorghum, rice, and soybean; and to assess the disease potential of *T. cucumeris* on rice.

MATERIALS AND METHODS

Etiology of *R. solani* from sorghum. Isolates of *R. solani* obtained from the leaves and sheaths of seven sorghum cultivars were compared with those from blighted rice and soybean leaves. The anastomosis group (AG) of three *R. solani* isolates obtained from sorghum leaves was determined by a procedure described by Parmeter et al (9). Isolates were paired with a set of test isolates (AG 1-4) donated from Parmeter's collection by E. E. Butler of the University of California at Davis. One isolate from sorghum was also paired with isolates from blighted tissues of rice, soybean, signalgrass (*Panicum dichotomiflorum* Michx.), and "coastal" bermudagrass (*Cynodon dactylon* (L.) Pers.).

Perfect state of the pathogen on sorghum. Hymenia of *T. cucumeris* actively producing basidiospores were frequently found associated with diseased tissues in sorghum fields in 1975. A method was developed to obtain single-basidiospore cultures from hymenia produced on host tissues. A strip of leaf tissue containing the hymenium was taped to the inner surface of a petri dish lid, and the basidiospores were allowed to drop onto the surface of 2% water agar in the petri dish bottom. At 12-hr intervals, the lids were repositioned over fresh water agar. Plates containing basidiospores were flooded with 0.01% streptomycin sulfate in sterile distilled water. Spores were dispersed in the liquid

by gently rubbing the agar surface with a sterile glass rod. The liquid containing basidiospores was drawn into a pipette and immediately distributed to the surface of several potato-dextrose agar (PDA) plates. The basidiospores transferred to PDA were allowed to germinate overnight, and single, isolated, germinating basidiospores were then located by viewing the plates through a dissecting microscope. Basidiospores were removed from the plate by cutting a piece of PDA out around them and placing them on fresh PDA. All isolates collected were maintained at 16 C on PDA amended with casein hydrolysate and yeast extract at 0.5 g/L each.

One hundred randomly selected basidiospores from six hymenia produced on sorghum leaves and sheaths in the field were measured with an optical micrometer. Sixty-eight single-basidiospore isolates were transferred to fresh PDA plates and incubated without light for 14 days at 28 C. The cultural characteristics of three replicated plates of each isolate were examined and compared with heterokaryotic isolates from diseased leaves.

Virulence of *R. solani* isolates from sorghum to rice. Twelve single-basidiospore isolates differing in cultural appearance on PDA were selected for tests of virulence to eight rice cultivars that showed a wide range of disease reactions from susceptible to resistant to the rice sheath blight isolate, LR172. This isolate was obtained as a mass transfer from a diseased rice sheath in 1972 and had been used exclusively in extensive cultivar screening tests for resistance to sheath blight (M. C. Rush and T. Masajo, unpublished data). Also included in the experiment was isolate LR14675, obtained from blighted sorghum leaf tissue and similar to LR172 in cultural characteristics on PDA.

Rice seedlings were grown in steam-sterilized field soil, washed sand, and peat moss (2:1:1, v/v/v) in galvanized metal flats (20 × 30 × 8 cm deep) arranged in greenhouse benches (6.1 × 1.2 m) lined with polyethylene sheets. Eight half-rows were marked across each flat, and about 1 g of seed of each of the eight cultivars of rice was planted in each row. The benches were flooded with water to a depth of 3.5 cm above the soil level. When the seedlings were 2 wk old, 13-13-13

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fertilizer (N-P₂O₅-K₂O) was applied at the rate of 449 kg/ha, and ammonium sulfate was applied at the rate of 225

kg/ha. Inoculum consisted of a moist, sterile rice hull:rice grain (2:1, v/v) mixture inoculated, then incubated for 2 wk

or until the medium was completely infested. Seedlings were inoculated with the isolates 40 days after sowing by distributing about 150 ml of inoculum uniformly over the seedlings in each flat. A chamber covered with polyethylene plastic and designed to maintain high humidity and temperature was constructed over each bench and removed 9 days after inoculation. At 10 and 19 days, the cultivars were given a disease rating on a scale of 0-9, where 0 = 0-10% and 9 = 91-100% of foliage infected.

The experimental design was a completely randomized block with each of 15 flats inoculated with one of the 14 isolates. One flat received sterile culture media. Each block was replicated three times, and the experiment was repeated twice. A virulence mean computed as the mean disease reaction from the eight cultivars was assigned to each isolate. Coefficients of concordance, according to the method of Kendall (3), were determined for the rankings of cultivar reactions to the *R. solani* isolates.

In a similar test, the reactions of eight rice cultivars to four heterokaryotic isolates from sorghum were compared with reactions of these cultivars to 18 isolates of *R. solani* from rice.

RESULTS AND DISCUSSION

During the 1975, 1976, and 1977 growing seasons, plants in several sorghum fields in Louisiana were observed with severe blighting of sheaths, leaf blades, and stems. The symptoms, similar to those described by Bell et al (1), were expressed as large, white-centered lesions, sometimes appearing as a series of 2- to 8-cm-wide, reddish brown, bordered bands across the leaf (Fig. 1). Leaf sheaths and stems developed brown-bordered, oval lesions with white centers. Sclerotia measuring 1-6 mm in diameter were produced at the margins of leaf, sheath, and stem lesions. Cultural characteristics of *R. solani* isolates from blighted foliage of sorghum cultivars Pioneer, Roma, Ramada, Rio, Funk G-516, Golden Grain, and AKS-614 were similar to each other and to isolates from blighted rice and soybean tissues. The sorghum isolates anastomosed with AG-1, the same AG to which foliar-blighting *R. solani* isolates from rice and soybean belong (7) (Table 1).

The teleomorphic state of the foliar-blighting *R. solani* was found in several sorghum fields. Hymenia were located on the lower leaf surfaces of apparently healthy sorghum tissue but adjacent to the large, white-centered lesions (Fig. 2). Most hymenia were roughly circular and very large, covering an area of 16 cm² or more. The hymenia typically consisted of racemes of barrel-shaped basidia that were wider than the supporting hyphae. Basidiospore production by hymenia on leaves brought to the laboratory continued for 6-11 days.

Table 1. Anastomosis reaction of *Rhizoctonia solani* isolates from blighted sorghum foliage

Sorghum isolate	Test isolate ^a	Anastomosis reaction ^b
LR14675 (Funk G-516)	AG-1 (pine seedling)	+
LR14675	AG-2 (Gypsophila)	-
LR14675	AG-3 (bean seedling)	-
LR14675	AG-4 (conifer seedling)	-
LR14675	LR172 (rice)	+
LR14675	LR6173 (soybean)	+
LR14675	LR16974 (signalgrass)	+
LR14675	LR20075 (coastal bermudagrass)	+
LR1476 (Rio)	AG-1	+
LR1576 (Ramada)	AG-1	+

^aTest isolates were obtained from the hosts indicated. Isolates representing anastomosis group (AG) 1-4 were donated by E. E. Butler, University of California, Davis.

^bPositive indicates that at least three anastomoses were observed.

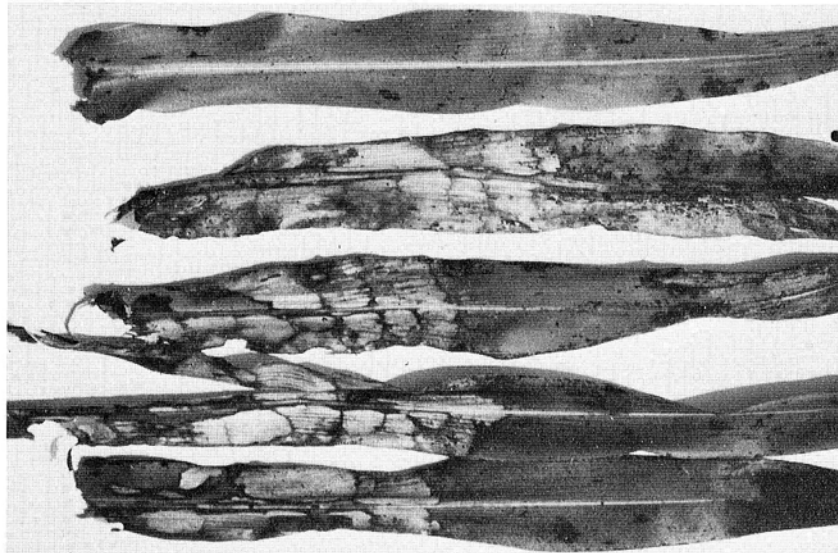


Fig. 1. Typical leaf symptoms on sorghum naturally infected by *Rhizoctonia solani*. Symptoms on severely infected foliage appear as a series of large, white, coalescing lesions that produce a banding effect similar to symptoms on rice leaves susceptible to *R. solani*.

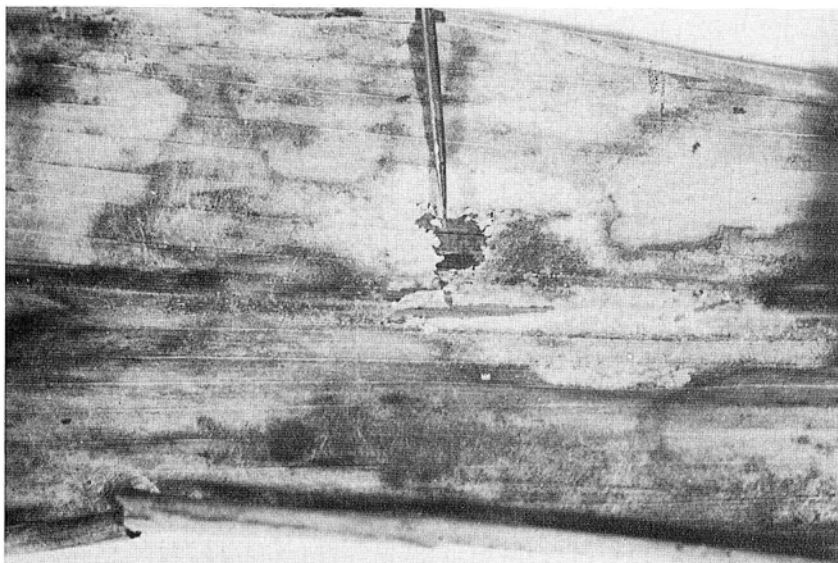


Fig. 2. White area adjacent to the lesions on blighted sorghum leaf is the hymenium of *Thanatephorus cucumeris*. All hymenia observed were superficial. A portion of the hymenium is scraped away at the point of the needle to show that it is superficial and that apparently healthy tissue lies beneath it.

Germination of basidiospores was observed to be less than 24 hr after placing hymenia over water agar in petri plates. Almost all basidiospores germinated with a single, nonseptate germ tube emerging from the end opposite the apiculus. A few, however, germinated by repetition. Anastomoses were occasionally observed between septate germ tubes growing from different basidiospores.

Basidiospore morphology was typical of *T. cucumeris* (13). The basidiospores were smooth, prominently apiculate, oblong to broad-ellipsoid, unilaterally flattened, and widest toward the distal end. Mean dimensions of 100 basidiospores were $7.02 (\pm 2.62) \times 8.72 (\pm 2.12) \mu\text{m}$, which was within the range reported for basidiospores from *T. cucumeris*.

The cultural characteristics of isolates of *R. solani* from diseased sorghum, soybean, and rice foliage were identical. All isolates produced abundant, scattered, tan-to-brown sclerotia after 1 wk. No aerial mycelium was observed, and with each isolate the medium became tan and eventually brown. The cultural characteristics of 68 single-basidiospore isolates from hymenia on sorghum varied from an abundance to the absence of aerial mycelium, brown pigmentation, and sclerotia. Sclerotial size and mycelial growth rates also varied between isolates. None of the single-basidiospore cultures resembled heterokaryotic isolates obtained from diseased sorghum foliage in all characteristics.

Isolate LR14675, obtained from sorghum tissue, was less virulent on six of the rice cultivars than LR172 obtained from rice tissue, but more virulent than any of the single-basidiospore isolates obtained from sorghum with two

exceptions (Table 2). The virulence mean of the single-basidiospore isolates on the eight rice cultivars varied from 1.5 to 6.1. Three isolates with low virulence on rice could not be reisolated from lesions on foliage.

Although virulence among the single-basidiospore isolates to the rice cultivars differed, the cultivars seemed to be ranked similarly in susceptibility and resistance to *R. solani* and were similar to rankings determined previously for LR172 in the field under less disease

pressure (Fig. 3). For example, Lebonnet was a very susceptible rice cultivar to isolate LR172 from rice and was also very susceptible to single-basidiospore isolate LR12975 from sorghum, which had a low level of virulence on rice. Cultivars of rice moderately resistant to LR172 exhibited resistant-type lesions with wide brown margins, and cultivars susceptible to LR172 exhibited susceptible-type lesions with narrow brown borders regardless of the isolate used. Coefficients of concordance (W) (3) were determined for the

Table 2. Reactions of eight selected rice cultivars to *Rhizoctonia solani* isolates LR172, LR14675, and 12 single-basidiospore isolates from sorghum in a greenhouse test for virulence

Isolate ^x	Mean disease rating ^y of cultivar								Virulence mean ^z
	Tetep	Taducan	Zenith	Nato	Saturn	Starbonnet	Lebonnet	Dawn	
LR172	8.25	7.83	7.83	8.50	8.67	8.50	8.33	8.50	8.30 g
LR14675	4.67	5.17	5.67	6.17	5.50	8.33	8.67	8.67	6.61 fg
LR12775	3.83	4.50	5.17	6.67	5.67	7.50	7.50	8.00	6.11 efg
LR13175	3.00	3.67	4.33	5.33	4.50	5.33	7.00	6.67	4.98 defg
LR12975	1.67	2.83	3.33	4.83	4.83	6.83	7.50	5.00	4.60 cdef
LR11975	3.83	4.17	3.33	4.50	4.50	4.67	5.33	5.33	4.46 bcdef
LR13275	2.17	2.50	2.83	2.67	1.83	3.35	4.50	2.83	2.80 abcde
LR1847	2.33	2.50	2.50	2.67	2.83	3.83	2.67	2.83	2.77 abcde
LR13375	1.67	2.33	1.83	2.17	2.17	4.33	4.33	3.00	2.73 abcde
LR16075	3.00	1.50	2.00	2.50	2.67	2.50	2.83	2.50	2.44 abc
LR13575	1.33	1.17	1.83	2.67	2.00	3.17	4.33	2.33	2.35 abc
LR18175	1.67	1.83	2.33	2.33	2.17	3.33	2.50	2.33	2.31 abc
LR15875	1.67	1.50	1.83	1.83	1.50	2.83	2.17	2.67	2.00 ab
LR15975	1.33	1.17	1.17	1.50	1.17	1.83	2.17	1.67	1.50 a
Cultivar mean ^z	2.89 a	3.05 a	3.28 ab	3.88 abc	3.57 abc	4.75 bc	4.99 c	4.45 abc	

^xIsolates LR172 and LR14675 are heterokaryon isolates obtained from rice and sorghum. All other isolates are single-basidiospore cultures obtained from *Thanatephorus cucumeris* hymenia on sorghum.

^yBased on a 0-9 scale of increasing susceptibility. Each number represents the mean of 12 determinations. Cultivars are listed in order of increasing susceptibility as determined by their reaction to LR172. Conditions in the greenhouse test were so favorable for disease development that resistance to LR172 was not expressed as normally observed in the field.

^zTreatments followed by the same letter within a column or row do not differ significantly according to Duncan's multiple range test ($P = 0.05$).

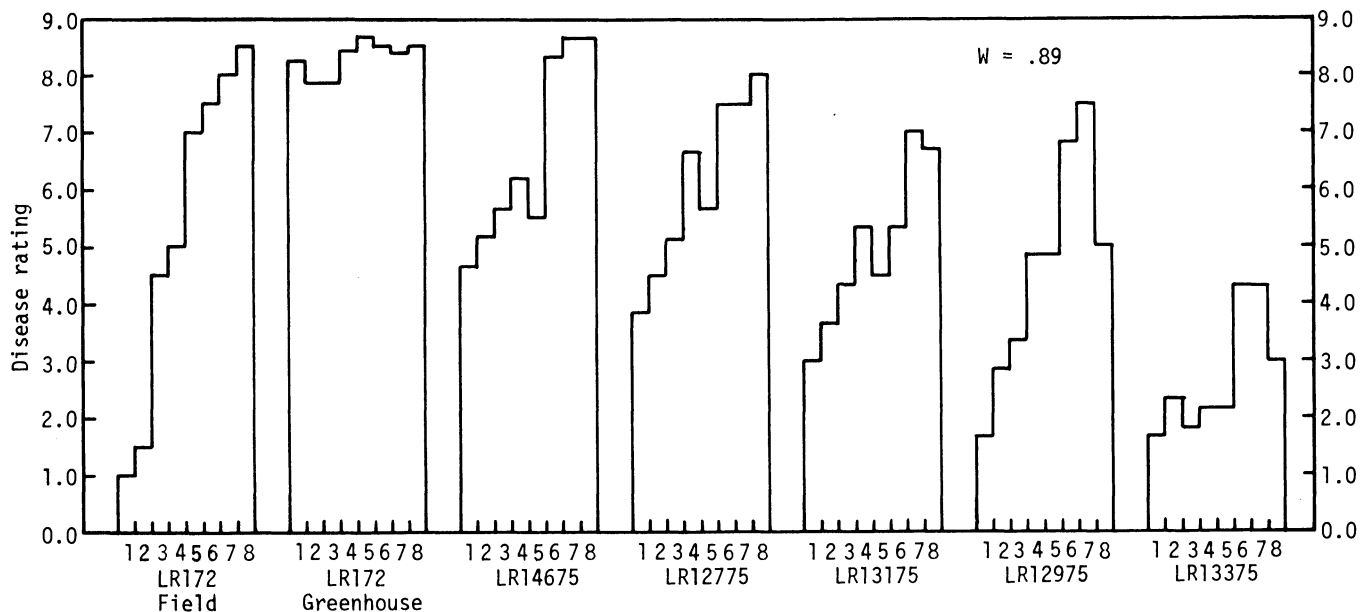


Fig. 3. Response in the greenhouse of eight rice cultivars to infection by single-basidiospore isolates of *Rhizoctonia solani* obtained from hymenia on sorghum. Included are *R. solani* field isolates from sorghum (LR14675) and rice (LR172). Rice cultivars one through eight are Tetep, Taducan, Zenith, Nato, Saturn, Starbonnet, Lebonnet, and Dawn, respectively. Rating is based on 0-9 scale in order of increasing susceptibility to *R. solani*. Included for comparison is the varietal response to LR172 in the field. Each rating represents the mean of 12 determinations. The W represents the coefficient of concordance of the rice cultivars to isolates and is significant at the 1% level of probability.

reactions of rice cultivars to the single-basidiospore and heterokaryotic isolates ($W = 0.89$), and the hypothesis of nonequivalence of ranks of the isolates by cultivars was rejected at the 1% level. These experiments suggested that the genes conditioning resistance to *R. solani* in rice cultivars were effective against the different pathogen genotypes tested.

The virulence mean of heterokaryotic isolates of *R. solani* from rice and sorghum, as determined with eight cultivars of rice, ranged from 4.89 to 6.79 for sorghum isolates and 5.14 to 8.29 for rice isolates (*data not shown*). The differences were not significant.

Numerous attempts to produce the teleomorphic state of an aerial *R. solani* in vitro have been unsuccessful. Consequently, the infectivity, survival, and soil colonization of single basidiospores were not studied. The lack of variant forms in more than 100 heterokaryon *R. solani* isolates collected from diseased tissues of rice, soybean, and sorghum suggests that homokaryotic basidiospores produced in nature anastomose to form heterokaryons sometime prior to infection, or they do not survive. Papavizas (8) has demonstrated that some single-basidiospore isolates are good competitive saprophytes in soil, especially when the basidiospores are produced from strains of *R. solani* possessing high survival capability. When Olsen et al (5) compared the survival of homokaryotic, single-basidiospore isolates of *T. cucumeris* with heterokaryotic mycelium, they found that some homokaryons survived over a 12-mo period and produced higher populations than the parents.

The low virulence to eight rice cultivars found in the single-basidiospore homokaryons suggested that high virulence in an isolate requires the presence of more

than one kind of nucleus. Vest and Anderson (16) found that when low-virulence, single-basidiospore homokaryons of *R. solani* from flax (*Linum usitatissimum* L.) are paired and anastomosed successfully, the heterokaryons formed are usually as virulent as or more virulent than either homokaryon, and in some cases more virulent than the parent of the homokaryons. Further research is needed to determine the virulence to rice and sorghum of synthesized heterokaryons from homokaryotic basidiospores of *R. solani* strains causing foliar blighting.

This research demonstrated that the same population of *R. solani* in Louisiana that causes sheath blight of rice and aerial blight of soybean also caused a sheath blight on sorghum. Sorghum is not at this time a major crop grown in Louisiana. However, inoculum produced on diseased sorghum may increase the potential for disease in fields rotated with rice or soybeans. The existence of the perfect state may also provide additional means of dissemination of the pathogen. Echandi (2), working in Costa Rica, reported that basidiospores of the teleomorph are the source of secondary inoculum for web blight of common bean (*Phaseolus vulgaris* L.). Aerial dissemination of basidiospores in sorghum fields was also suggested by observations of areas in fields where the disease developed on aerial leaf parts with no evidence of mycelium or symptoms on lower leaf sheaths or stems. The rapid spread of sheath blight of sorghum when conditions are warm and humid may be explained by production and dissemination of basidiospores produced by *T. cucumeris* on sorghum.

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