Infection Cushion Formation on Rice Sheaths by *Rhizoctonia solani*

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ABSTRACT

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Replicas were made of outer sheath surfaces of rice cultivars which were resistant or susceptible to rice sheath blight caused by *Rhizoctonia solani*. Lobate appressoria and infection cushions, which are infection structures formed by the fungus on sheath surfaces, were not formed on the replicas. When sheath tissue from rice cultivars was placed under collodion membranes, both types of infection structures formed on the membranes over tissue from susceptible cultivars. Cultivars varying in levels of resistance to sheath blight were grown under identical greenhouse conditions. Abundant wax deposits were observed on the outer sheaths of resistant cultivars. No wax deposits were observed on the outer sheaths of susceptible cultivars. Cultivars intermediate in resistance had varying amounts of wax deposits on their outer sheaths. Removal of wax deposits from resistant cultivars with chloroform, followed by inoculation with *R. solani*, resulted in a susceptible reaction. An exogenous supply of glucose and 3-O-methylglucose reduced lesion development in rice seedlings. The apparent mode of action of 3-O-methylglucose was to inhibit infection cushion formation.

The prepenetration activities of *Thanatephorus cucumeris* (Frank) Donk [*Rhizoctonia solani* (Kühn), *Pellicularia sasakii* (Shirai) S. Ito], and the factors which control them are complex. Included in these activities are the formation of infection cushions and/or lobate appressoria (7–9). DeSilva and "Wood" (6) indicated that infection cushion formation was due to a contact stimulus. Other evidence suggests that infection cushion formation was controlled by host exudates (8,9,16,24).

Nutrition of *R. solani* prior to penetration plays an important role in pathogenesis (23). It has been demonstrated that both glucose and 3-O-methylglucose reduce lesion development on cotton hypocotyls (22).

The sheath blight disease of rice, which is caused by *R. solani*, is a major disease in the southern United States and, in recent years, it has been increasing in importance worldwide (2,18–20). Therefore, a greater understanding of how this pathogen infects rice and methods for inhibiting its pathogenesis are needed.

The purpose of the present study was to investigate the nature of infection cushion formation by *R. solani* on rice cultivars with differing levels of resistance to sheath blight.

MATERIALS AND METHODS

Fungus isolate and rice cultivars. The virulent isolate LR 172 of *R. solani* was isolated from naturally infected Lebonnet rice in Louisiana. The isolate was identified as belonging to anastomosis group AG-1 (17). The fungus was maintained on an autoclaved rice seed/rice hull mixture (2:1, v/v). The rice cultivars used and their reactions to sheath blight were: Taducan and Tetep (resistant); Saturn and Zenith (moderately susceptible); Dawn (susceptible); and Labelle, Lebonnet, and Bluebelle (very susceptible) (10).

Cultivar growth conditions. For all experiments, rice seed was surface sterilized in 0.525% sodium hypochlorite for 2 min, rinsed in sterile distilled water (SDW), and placed in a small amount of SDW for 24 hr at 28 C to allow imbibition of water. The seeds were planted in a silt loam soil in 20.3-cm-diameter pots. A 12-hr day temperature of 30 ± 5 C and a night temperature of 26 ± 3 C were maintained in the greenhouse.

Surface replicas. Sheath pieces from cultivars Taducan, Tetep, Labelle, Lebonnet, and Bluebelle were excised at the maximum tillering stage of the plant. The method used to make surface replicas was adapted from Wynn (25) and Clark and Lorbeer (5). Silicone rubber was spread on the outer epidermal surface. The rubber-coated tissue was placed between a flat surface and a glass slide held with a 100-g weight, and left undisturbed overnight. The plant material was then removed from the replica. The replica was rinsed twice in chloroform for 10 sec, washed 2 hr in distilled water, and cured for 1 hr at 180 C. Both fingernail polish and polystyrene were used to make positives. The first positives that were made were discarded to eliminate any leaf surface impurities which might have been carried over on the rubber negatives.

Collodion membranes. This procedure was carried out as described by Flentje et al (9). To form the membranes, 0.4 g of Parlodion was dissolved in a mixture containing 85 ml ether and 15 ml absolute alcohol. Amyl acetate was used as an alternate solvent. The solution was then spread on a glass plate and the solvents were allowed to evaporate. Pieces of collodion large enough to fit over plastic O-rings were cut out on the glass. The collodion was then floated off the glass plate by lowering the glass plate into water at an angle. A single membrane was placed over each O-ring. The fungus was placed on top of the membrane with the plant material under it. The membrane was in contact with the plant material surface but did not assume its topography.

Effects of cuticular waxes. At the maximum tillering stage, plants were exposed to the following treatments: untreated; stems drawn through a pad of cotton; stems drawn through a pad of cotton soaked with chloroform; and stems drawn through a pad of cotton soaked with water. Following each treatment the stem was inoculated with *R. solani*. Pieces of stem were excised 48 hr after inoculation, fixed in FAA with mercuric chloride (1 g $HgCl_2$ per 10 ml FAA) at 4 C, rinsed in distilled water, and dehydrated in acidified DMP (2,2-dimethoxypropane + HCl). DMP was used because it did not solubilize wax deposits. The dehydrated tissue was critical-point dried in liquid CO_2, mounted on aluminum stubs and coated with 20 μm (200 Å) of gold-palladium in a sputter coater. Observations were made with an Hitachi S-500 scanning electron microscope operated at 25 kV.

Effects of exogenous nutrients on disease development. To facilitate microscopic observation, the following process was adapted from Anderson and Stretton (1) and Weinhold and Bowman (22). Rice seedlings were grown in washed and ignited sand for 7 days in an environmental chamber with a 12-hr day at 28 C and a 12-hr night at 25 C. The plants were watered with Hoagland's nutrient solution (21). The seedlings were removed from the sand and the roots were washed with distilled water. Two
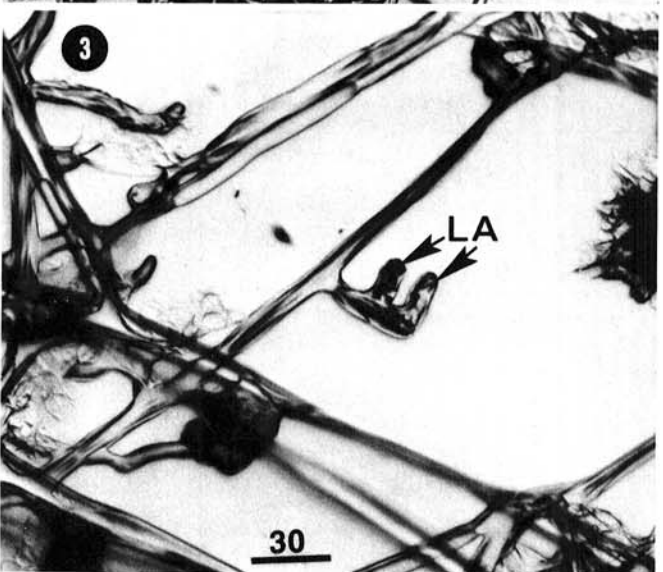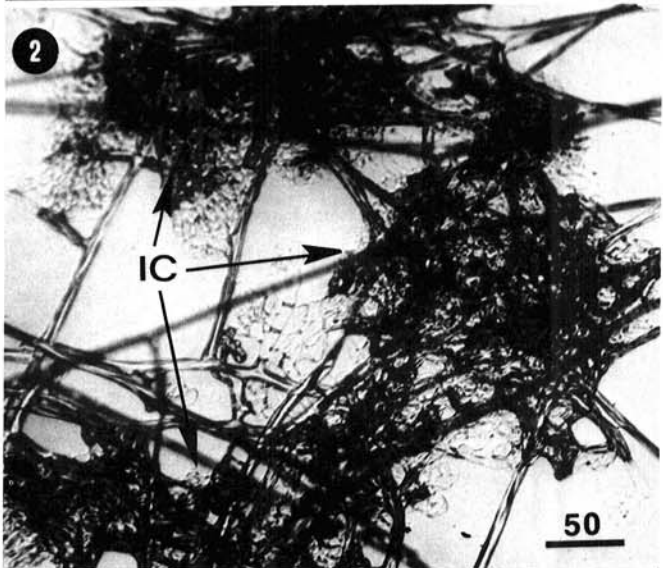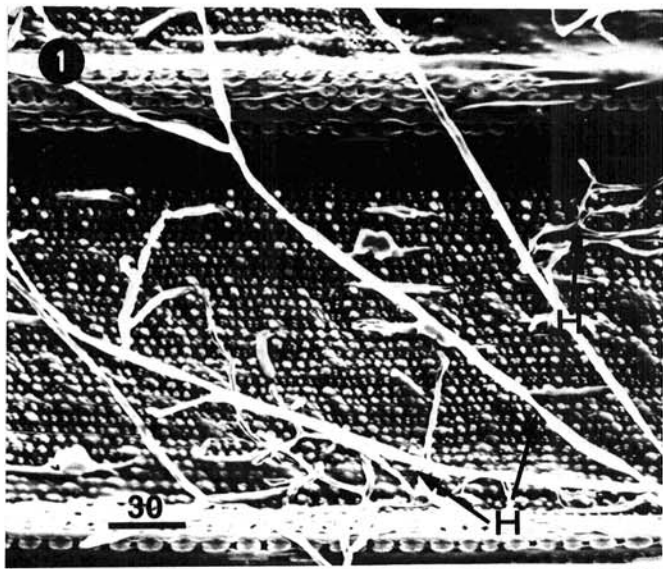
Figs. 1–3. Scanning electron and phase-contrast light micrographs of development of *Rhizoctonia solani* on artificial surfaces. **1,** Growth of *R. solani* on polystyrene rice sheath surface replica. Note random growth pattern. **2,** *R. solani* infection cushions produced on collodion membrane over sheath surface of Bluebelle cultivar. **3,** *R. solani* lobate appressoria produced on collodion membrane over Bluebelle cultivar. Abbreviations: H = hyphae, IC = infection cushion, and LA = lobate appressorium. Calibration bars are in micrometers (μm).
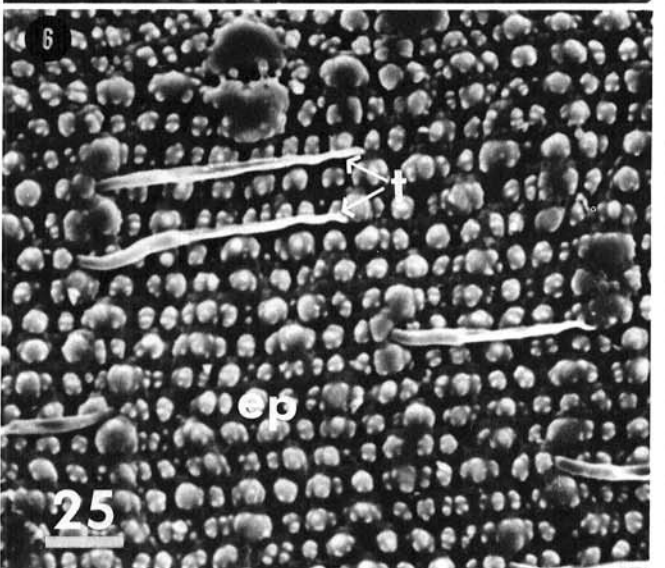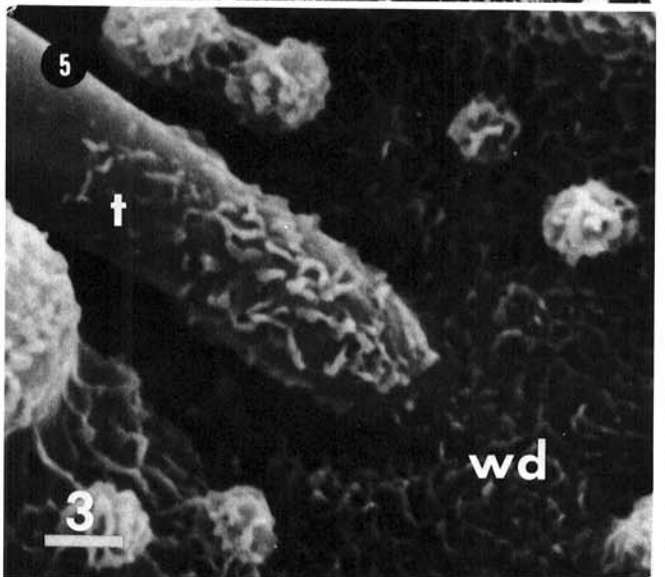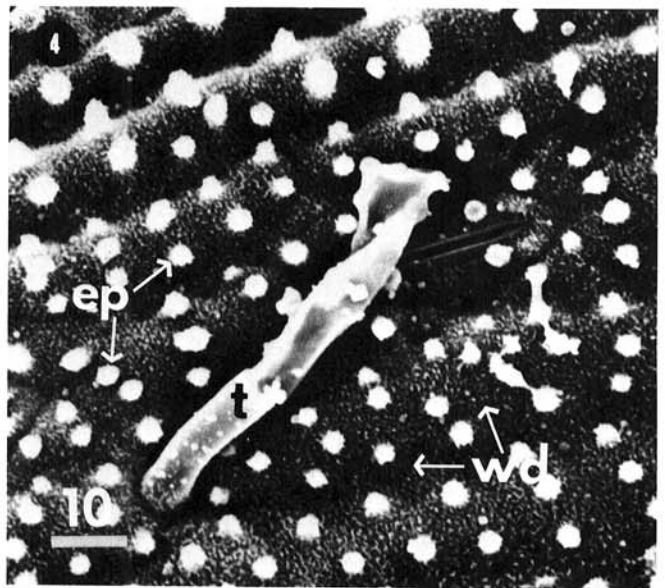
Figs. 4–6. Scanning electron micrographs of wax production on outer epidermal sheath surface of different rice cultivars. **4,** Outer sheath surface of resistant rice cultivar Taducan showing wax deposits. **5,** High magnification of wax deposits on Taducan. **6,** Outer sheath surface of susceptible rice cultivar Bluebelle showing absence of wax deposits. Abbreviations: ep = epidermal cell projections, t = trichome, and wd = wax deposits. Calibration bars are in micrometers (μm).

seedlings were attached to a microscope slide with two rubber bands and placed vertically in the sand. A piece of rice seed:rice hull medium colonized by *R. solani* was placed on the sand approximately 1 mm from each of the seedlings. The seedlings were then returned to the environmental chamber. Exogenous nutrients were supplied by moistening the sand with 40 ml of solution of either 3-O-methylglucose (MEG) or glucose.

RESULTS

Fungal development on sheath surface replicas. Development of *R. solani* hyphae on replicas of rice sheaths was unlike that on the rice sheath itself. The hyphae did not follow the junction of the anticlinal walls of the underlying epidermal cells. Instead, they grew randomly on the sheath replica surface (Fig. 1). No infection structures formed on the replicas. This was true whether the replica was made from the sheath of a resistant (cultivars Taducan or Tetep) or susceptible (cultivars Labelle, Lebonnet, or Bluebelle) rice plant.

Infection structures on collodion membranes. *R. solani* propagules placed on collodion membranes over stem pieces from resistant, intermediate, and susceptible cultivars exhibited typical hyphal proliferation and appressorium formation. On the membranes covering stem pieces of intermediate and susceptible cultivars, there was hyphal proliferation and the formation of typical lobate appressoria and infection cushions (Figs. 2 and 3).

Effects of cuticular wax deposits. When the cultivars were grown in the greenhouse simultaneously, processed identically, and observed in the SEM, it was seen that the resistant cultivars had an abundance of cuticular wax deposits on the outer sheath surface (Figs. 4 and 5). Susceptible cultivars had no wax deposits on the outer sheath surface (Fig. 6). Cultivars with intermediate susceptibility had varying amounts of cuticular wax deposits.

When wax deposits were removed from the resistant cultivars with chloroform and the plants were inoculated with *R. solani,* both lobate appressoria and infection cushions were produced, even though these cultivars previously had not supported infection cushion formation. The chloroform treatment did not affect the susceptible cultivars. Rubbing with dry cotton or water-soaked pads had no effect on any cultivar tested.

Effect of exogenous glucose and MEG on the aggressiveness of *R. solani.* The presence of an exogenous supply of MEG and glucose resulted in a decided reduction in lesion development on rice seedlings (Table 1). Lesion development was completely suppressed by MEG at 5.2 mM on cultivars Tetep and Zenith and 10.4 mM on Bluebelle.

Glucose also effectively reduced disease severity, but the concentrations needed were higher than those of MEG. A decrease in lesion development was noticeable on all three cultivars at 20.4 mM and higher.

When MEG and glucose were combined at 10.4 and 10.2 mM, respectively, there was a complete repression of lesion development at both 48 and 72 hr. *R. solani* proliferated to a higher degree on the seedlings and surrounding glass slide of both the glucose and MEG treatments than on the water controls.

When Tetep, Zenith, and Bluebelle cultivars were examined at various time intervals after inoculation, both infection structures were produced on the water controls of Bluebelle and Zenith approximately 18 hr after inoculation. On the Tetep control, lobate appressoria were produced at approximately the same time. In the presence of a solution containing 40.8 mM glucose, infection structure formation was delayed until 30 hr after inoculation on all three cultivars. With a solution of 5.2 mM MEG, infection cushion formation was completely suppressed up to 72 hr after inoculation on Zenith and Bluebelle. Lobate appressoria were not produced on these two cultivars until approximately 60 hr after inoculation.

DISCUSSION

The results presented indicate that the growth of *R. solani* on rice sheaths and the formation of infection structures by this pathogen were not controlled by a contact stimulus. Growth of *R. solani* on rice sheath replicas was different from that on rice sheaths. The growth patterns on replicas made both from resistant or susceptible cultivars were similar. There is strong evidence in the literature which suggests that infection cushion formation by *R. solani* is induced by host exudates (8,9,16,24). However, DeSilva and Woods (6) were able to produce infection cushion-like structures on washed strips of host cuticle and epidermis without the addition of the exudate.

The formation of infection cushions by *R. solani* on collodion membranes has been demonstrated (9). It has been suggested (13) that the disease severity rating of rice cultivars to sheath blight was highly correlated with the number and type of infection structures produced by the pathogen on the cultivar. On cultivars with low disease severity ratings, lobate appressoria were formed, but infection cushions were not. On collodion membranes placed over susceptible and intermediate cultivars, both infection cushions and lobate appressoria were formed. On the membranes placed over resistant cultivars, only lobate appressoria were formed. This provides preliminary evidence that exudate controls formation of these structures and has a differential effect on infection structure formation.

When rice cultivars with different disease severity ratings were grown under identical conditions, they varied in the amount of wax deposits on the outer sheaths. Cultivars resistant to the disease had abundant wax deposits, cultivars intermediate in resistance produced varying amounts of wax deposits, and susceptible cultivars produced no wax deposits. After removal of the deposits from resistant cultivars with chloroform, infection cushions were formed. This phenomenon was previously reported by the authors (14), but its cause is not known. The wax deposits may restrict the movement of an infection cushion elicitor to the sheath surface. However, the deposits themselves or substances associated with them may be inhibitory to infection cushion formation. Wax associated substances were shown to contribute to the antifungal properties of plants (3,4,12,15). Since wax formation and structure are greatly influenced by environmental factors, particularly temperature and light intensity (11), it is possible that given different environmental conditions, the cultivars which did not form waxes may do so.

The nutritional status of *R. solani* hyphae prior to penetration had a decided effect on aggressiveness of the pathogen. Weinhold and Bowman (22) suggested that the effect of glucose on the reduction of lesion development on cotton hypocotyls was caused by pectinase repression. They further stated that the reduction lesion development by MEG was caused by the inhibition of infection cushion formation. These effects on cotton hypocotyl infection also appear to be important in *R. solani* infection of rice sheaths. An application of 5.2 mM MEG can suppress infection cushion formation even on the cultivar Bluebelle, which was highly susceptible to infection.

TABLE 1. Influence of exogenous glucose and 3-O-methylglucose (MEG) on lesion production[a] on rice by *Rhizoctonia solani*

Chemical concentration in solution used to moisten sand (mM)	Cultivars and time after inoculation					
	Tetep		Zenith		Bluebelle	
	48hr	72hr	48hr	72hr	48hr	72hr
MEG						
0.13	26.8	34.2	30.4	77.8	39.2	82.2
0.26	26.1	35.7	39.4	73.2	47.2	85.1
2.6	0.0	0.9	1.8	3.7	6.1	10.3
5.2	0.0	0.0	0.0	0.0	0.0	0.3
10.4	0.0	0.0	0.0	0.0	0.0	0.0
Glucose						
5.6	28.1	41.3	37.7	77.3	35.2	89.1
10.2	26.1	35.5	29.4	61.4	30.0	83.4
20.4	2.9	8.9	6.4	16.5	12.3	14.3
40.8	1.8	1.9	2.5	3.5	4.5	4.9
81.6	0.0	0.5	1.8	1.8	3.8	4.1
MEG + glucose						
(10.4 + 10.2)	0.0	0.0	0.0	0.0	0.0	0.0
Water check	27.0	38.3	33.5	64.4	41.5	78.1

[a] Mean lesion area (mm)^2.

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