Relation Between Infection by *Rhizoctonia solani* and *R. oryzae* and Disease Severity in Rice

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ABSTRACT

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Histological aspects of infection by *Rhizoctonia solani* and *R. oryzae* on rice cultivars differing in resistance levels were found to be identical. Both pathogens formed two infection structures, infection cushions and lobate appressoria. A highly significant correlation was detected between the formation of infection cushions and lobate appressoria (r = 0.977). Penetration pegs produced from these structures penetrated the plant surface directly. Stomatal penetration was infrequent and no infection structures were observed. Initial penetration occurred on the outer surface of the rice sheath. There were highly significant correlations between disease severity ratings of the cultivars and both infection structure formation (r = 0.994) and culm invasion (r = 0.935). The fungi failed to penetrate past the outermost sheaths of cultivars having low disease severity ratings and thus did not colonize the culms.

Sheath blight of rice (*Oryza sativa* L.) which is caused by *Thanatephorus cucumeris* (Frank) Donk (=*Rhizoctonia solani* [Kühn], *Pellicularia sasakii* [Shirai] S. Ito), is an economically important disease in the southern United States (1,15,18). Worldwide, among the fungal diseases of rice, it is second in importance only to blast (7,19,20). The sheath spot disease of rice, which is caused by *Rhizoctonia oryzae* Ryker and Gooch, has been reported only in the southern U. S. and southeast Asia, and is of minor economic importance (1,14,16).

Histological studies of sheath blight have shown that the fungus usually enters the plant through the inner sheath surface by means

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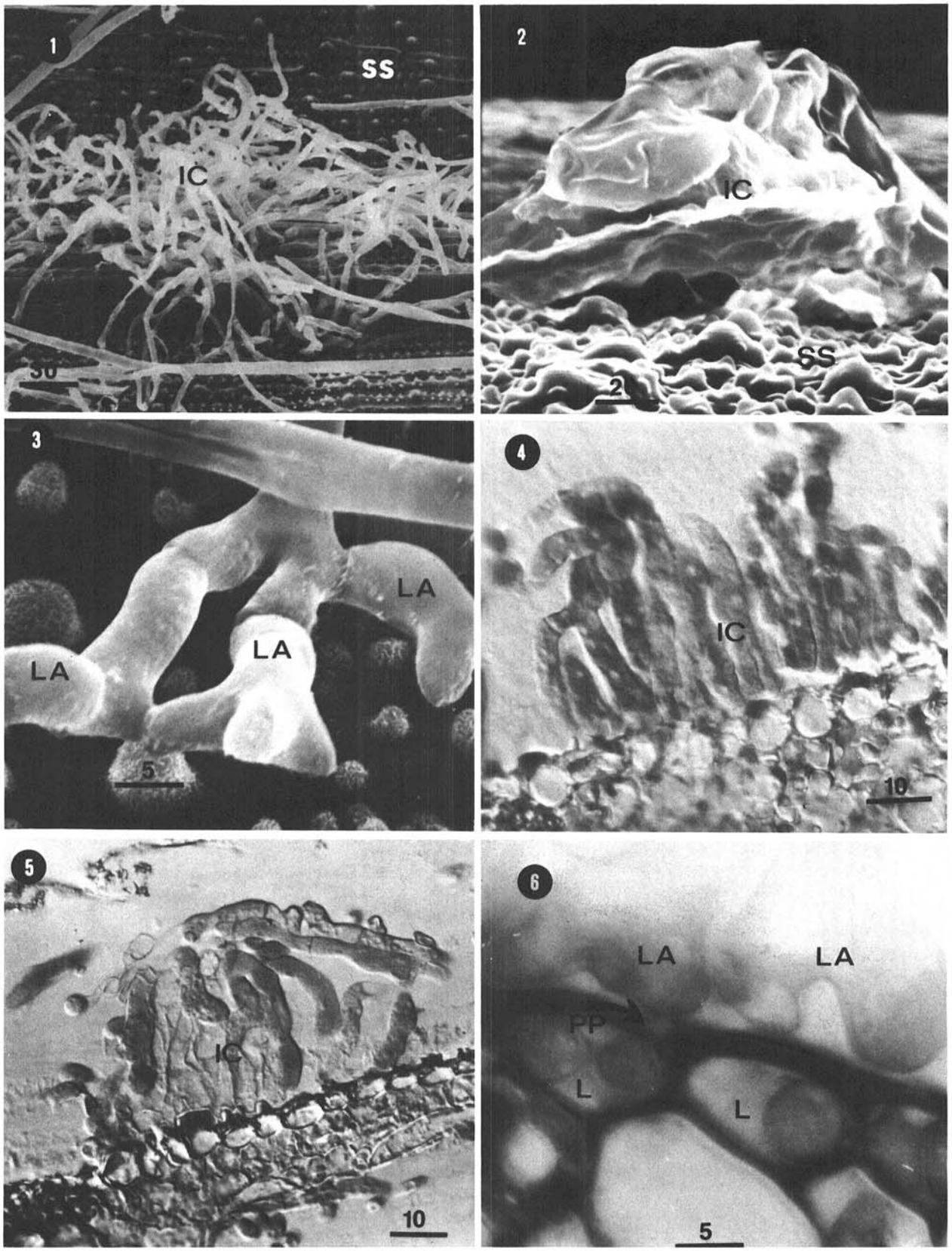


Figs. 1-6. Interference-contrast light and scanning electron micrographs of infection structures of *Rhizoctonia solani* and *R. oryzae*. Figs. 1 and 2: infection cushion of *R. solani* at **1,** an early stage of pathogenesis and **2,** a later stage of pathogenesis. **3,** Lobate appressoria of *R. solani*. Note that each appressorium forms several lobes at the apex. Figs. 4 and 5: cross sections through infection cushions of **4,** *R. solani* and **5,** *R. oryzae*. Both show cushion cells perpendicularly oriented to the sheath surface. **6,** Cross section through lobate appressoria showing penetration pegs and enlargement of hyphae in a cell lumen. Abbreviations: IC = infection cushion, L = cell lumen, LA = lobate appressorium, PP = penetration peg, and SS = sheath surface. Calibration bars are in micrometers.

of infection cushions and lobate appressoria (8,10). Spread of the fungus through tissue after penetration has not been investigated. A previous histological investigation of sheath spot showed that the prepenetration and penetration activities of *R. oryzae* and *R. solani* were identical (8).

Evidence indicates that in other diseases caused by *R. solani*, the ability of an isolate to establish infection may be determined at the infection cushion and penetration stages (4). Therefore, the objectives of the present study were to determine the relationships between mode of penetration, infection, and invasion by these pathogens and rice plant resistance.

MATERIALS AND METHODS

Isolates and cultivars. In all experiments, the virulent fungal isolates LR 172 of *R. solani* and LR 17174 of *R. oryzae* (both isolated from naturally infected Lebonnet rice from Acadia Parish, Louisiana) were used. *R. solani* isolate LR 172 was identified as belonging to anastomosis group AG-1 (13). Cultures were maintained on an autoclaved rice seed:rice hull mixture (2:1). The rice cultivars used were previously rated for disease severity to *R. solani* (9) and are listed in Table 1. All cultivars were field grown and inoculated with *R. solani*. In addition, plants of cultivars Taducan, Tetep, Saturn, Zenith, Dawn, Labelle, Lebonnet, and Bluebelle were grown in a greenhouse and inoculated with *R. solani* and *R. oryzae*.

Cultivar growth conditions. Seed to be used in greenhouse tests 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 imbibe water. 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. Seed used in field tests was not treated.

Inoculation and collection of tissue. Inoculum consisted of a mixture of sterilized rice seed and hulls colonized with either *R. solani* and *R. oryzae*. Plants were inoculated in the field and greenhouse by placing bits of inoculum on plants at the water line and at various other locations on the plant. A plastic humidity chamber was placed over greenhouse plants to enhance disease development.

Tissue pieces were excised 3 – 4 wk after inoculation from field grown plants after they had been rated for disease severity. Tissue pieces were excised from plants inoculated in the greenhouse at various times 1 – 48 hr after inoculation. Disease was allowed to progress on neighboring tillers of the same plant until a severity rating could be obtained. The disease severity rating was based on the size of the lesions and the percentage of tissue infected (9). All of the tissue pieces were examined and the number of infection structures were counted as described below. The disease severity rating was then compared with the number of infection structures.

Light- and scanning electron microscopy. Tissue pieces were cut (1.0–2.0 cm) and fixed in cold FAA (10 parts 37% formaldehyde: 50 parts 95% ethanol: five parts glacial acetic acid: 35 parts water, at 4 C) containing mercuric chloride (1 g $HgCl_2$ per 10 ml FAA) for 2 – 3 hr. The tissue pieces were transferred overnight through four changes of cold FAA without $HgCl_2$. The tissue was allowed to come to room temperature and rinsed three times in distilled water (10 min each) to remove the fixative. Dehydration was accomplished in acidified DMP (2,2-dimethoxypropane + HCl) followed by two changes of 100% acetone (12).

For light microscopy, tissue pieces were infiltrated and embedded in Spurr's plastic resin (17), sectioned at 2.5 μm with a glass knife, and stained with azure B blue. For scanning electron microscopy, tissue pieces were critical-point dried in liquid CO_2, mounted on aluminum stubs, and coated with 20 μm (200Å) of gold-palladium in a sputter coater. A Leitz Ortholux II, differential interference contrast microscope was used for light microscopy and an Hitachi S-500 operated at 25 kV was used for SEM observations. Reverse polarity was employed as needed for SEM observations.

Determination of culm invasion. To determine invasion into the culm, field- and greenhouse grown plants were collected 3 – 4 wk after inoculation. All the outer sheaths were stripped from the culms which were then cut into 2.5-cm pieces, surface sterilized for 5 min in 0.525% sodium hypochlorite, rinsed in SDW, and plated on 5% water agar.

RESULTS

The modes of penetration of *R. solani* and *R. oryzae* were identical; the following observations apply to both pathogens unless otherwise indicated.

Prepenetration activities. Following inoculation of the sheath surface, the hyphae grew longitudinally, generally following the junction of anticlinal walls of adjacent epidermal cells. After a short period of growth, (5 – 7 hr after inoculation) the hyphae produced side branches. These branches either continued to proliferate, becoming indistinguishable from the parent hypha or developed into one of two infection structures. One of these structures, the infection cushion, was a discrete aggregate of compacted hyphae (Figs. 1, 2). Infection cushions varied from 100 – 400 μm in diameter and 80 – 170 μm in height. Cushions were closely appressed to the plant surface, apparently without mucilaginous material. Individual hyphae within cushions were discernible in the early stages of pathogenesis (Fig. 1) but were obscure later (Fig. 2). Lobate appressoria also were formed. These were short, swollen branches which formed lobes at the apex (Fig. 3). They were also closely appressed to the plant surface without apparent mucilaginous material.

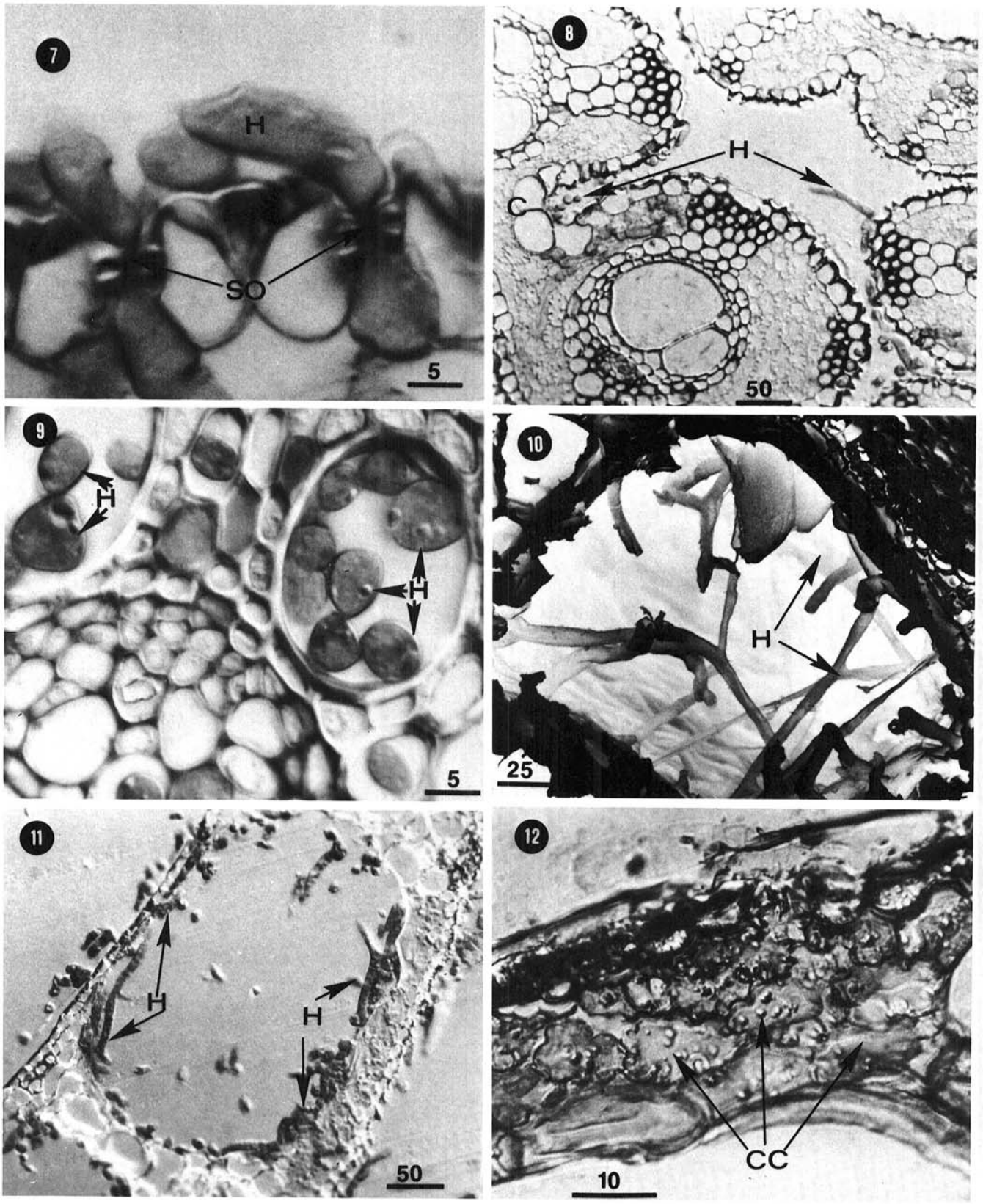
Penetration activities. Penetration from infection structures occurred either directly through or between epidermal cell walls, or through stomatal openings on the outer sheath surface. In direct penetrations, some of the cells of the infection cushions became oriented perpendicular to the plant surface. The bases of these cells became swollen and appeared similar to lobate appressoria (Figs. 4, 5). Both lobate appressoria and the perpendicular cells of cushions produced fine penetration pegs which penetrated the cuticle and cell wall and enlarged in the cell lumen (Fig. 6). Stomatal penetration was infrequent and did not involve the formation of either type of infection structure. Hyphae simply grew into the stomatal opening, constricted slightly, and regained the original diameter once inside the stomatal crypt (Fig. 7).

Postpenetration activities. Following penetration and re-

TABLE 1. Rice cultivars used in studying histological aspects of sheath blight caused by *Rhizoctonia solani* and sheath spot caused by *R. oryzae*

Level of resistance to *R. solani*[a]	Cultivar
Resistant	Taducan
	Tetep
	PI 7803097
	PI 7701052
	PI 7801020
Moderately resistant	PI 7801055
	PI 7603050
	PI 7803022
	Melrose
	Nova 66
Moderately susceptible	Saturn
	PI 7803020
	Zenith
	PI 7703008
	LA 110
Susceptible	Starbonnet
	Dawn
	PI 7801067
Very susceptible	Labelle
	Lebonnet
	Bluebelle
	PI 7801061
	PI 7801064

[a]Resistance level based on disease severity ratings as indicated in Hoff et al (9).



Figs. 7-12. Light (interference-contrast)-and scanning electron micrographs of stomatal penetration and invasion by *Rhizoctonia solani* and *R. oryzae* in rice plant tissues. **7,** Stomatal penetration by *R. oryzae*; note hyphal constriction through the stomatal openings and enlargement in the stomatal crypt. **8,** Hyphae of *R. solani* in the center of a rice culm. **9,** *R. solani* hyphae in cells of a vascular bundle. **10 and 11,** *R. solani* hyphae in intercellular cavities of sheath tissue (Fig. 10 has the polarity reversed in order to visualize hyphae in the cavity). **12,** Disfigured and collapsed cells of rice sheath around invading *R. solani* hyphae. Abbreviations: C = center of culm, CC = collapsed cells, H = hyphae, and SO = stomatal opening. Calibration bars are in micrometers.

establishment of normal hyphal diameter, the hyphae ramified inter- and intracellularly. Hyphal growth continued through to the center of the culm (Fig. 8), and on through to the opposite side. Longitudinal growth occurred through vascular bundles (Fig. 9) or more commonly, through intercellular cavities in sheaths (Figs. 10, 11).

Correlation of number of infection structures with disease severity ratings. The number of infection structures produced on plants inoculated with *R. solani* in the field and greenhouse were highly correlated with disease severity ratings (Fig. 13 A – C). In addition, as the number of infection cushions increased, the number of lobate appressoria also increased (r = 0.977). Infection cushions were formed on cultivars with disease severity ratings ranging 4 – 9, but none were formed on cultivars with ratings ranging 1 – 3.

Since a disease severity rating system for sheath spot has not been developed, it was necessary to select cultivars based on their sheath blight disease severity, to determine a possible correlation between infection structures of *R. oryzae* and disease severity. The number of infection structures counted on plants of selected greenhouse-grown rice cultivars inoculated with *R. oryzae* increased as the susceptibility of the cultivars to *R. solani* increased, but fewer structures were produced (Table 2).

Ability to invade the culm. Isolation of the pathogens was used as a measure of ability to invade the culm. The ability of *R. solani* to invade culms of a particular cultivar was highly correlated (r = 0.935) with the disease severity rating of that cultivar (Table 3). *R. oryzae* was never isolated from the culms of any cultivar. Histological observations of *R. solani* and *R. oryzae* on resistant cultivars showed that parenchyma cells within the first three to four layers of the outer rice sheaths became deformed and collapsed around invading hyphae (Fig. 12). The collapsed area corresponded to the sheath area having a reddish-brown discoloration around a restricted necrotic lesion, a symptom common on cultivars with low disease severity ratings. Collapse of cells around invading hyphae did not occur in susceptible cultivars.

DISCUSSION

The modes of penetration and infection of rice plants and other hosts by *R. solani* appears to be similar (2–6). After inoculation of the inner sheath surface with fungal mycelium, Hashioka and Okuda (8) studied the initial infection of both *R. solani* and *R. oryzae*, and Kozaka (10) investigated penetration by *R. solani*. Both investigations showed that the pathogens usually entered through the inner sheath surface. The present study indicates that initial penetration occurs through the outer sheath surface. This discrepancy in entry site could be due to the different methods of inoculation used. Under natural conditions, overwintered sclerotia and colonized crop debris in the soil float to the water surface and adhere to the outside of the sheath. We believe our method of placing pieces of rice seed and hulls colonized with the pathogen on

TABLE 2. Comparison of the mean number of infection structures produced by *Rhizoctonia solani* and *R. oryzae* on plants of selected rice cultivars.

Cultivar	Sheath blight rating (avg.)	Infection cushions[a] (mean no.)		Lobate appressoria[a] (mean no.)	
		R. solani	*R. oryzae*	*R. solani*	*R. oryzae*
Taducan	1.6	0.0	0.0	54.0	38.1
Tetep	1.7	0.0	0.0	52.5	37.6
Zenith	5.2	23.4	16.4	215.6	139.2
Saturn	5.4	23.8	15.0	222.4	134.7
Dawn	8.0	64.5	33.9	353.7	220.2
Bluebelle	8.4	73.4	42.3	384.6	287.0
Lebonnet	8.5	70.0	43.1	376.5	288.6
Labelle	8.7	71.2	48.6	377.7	294.4

[a]One hundred observations were made with each cultivar. All replicates were square-centimeter tissue pieces. *R. solani* data are pooled from greenhouse and field observations; *R. oryzae* data are from greenhouse observations only.

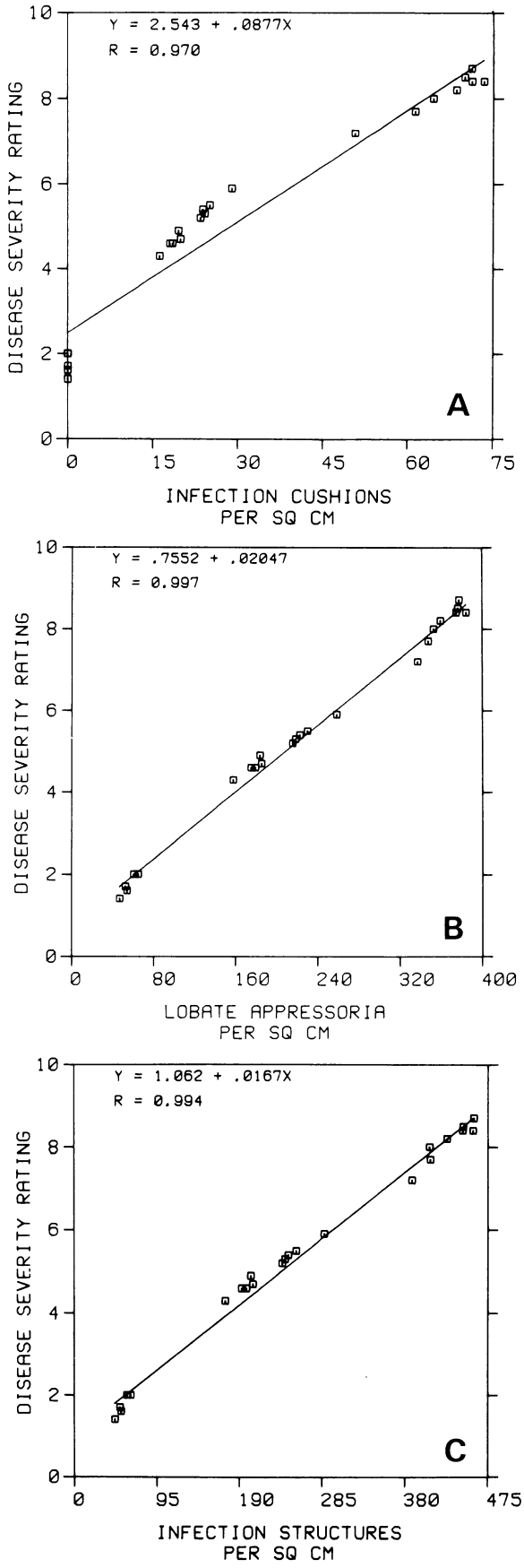


Fig. 13. Relation between disease severity ratings of field and greenhouse grown rice cultivars and: **A,** mean number of *Rhizoctonia solani* infection cushions; **B,** mean number of *R. solani* lobate appressoria, and **C,** mean number of *R. solani* total infection structures. To obtain the mean, 100 leaf tissue sample replicates (each a square centimeter) from plants of each cultivar were assessed for infection structures.

TABLE 3. Percentage of culms invaded by *Rhizoctonia solani* and correlation coefficient between disease severity rating (DSR) and culm invasion (CI)

DSR of cultivars	CI[a] (%)
0 – 3	4.0
4 – 6	46.5
7 – 9	91.4
DSR-CI = 0.935[b]	

[a]Seven replicates were used of each cultivar within each disease rating category. Data are pooled from greenhouse and field results.
[b]Correlation coefficient significant at $P = 0.01$.

the outer sheath surface more closely parallels the physical proximity of natural primary infection. The rapid upward movement of the fungus in the plant is probably facilitated by its growth in intercellular cavities in the rice sheath.

The disease severity rating system used to evaluate rice cultivars for resistance to sheath blight was based on the size of the lesions and the percentage of the plant tissue showing symptoms (9). The data presented here indicate that as the number of infection structures increases, there is a corresponding increase in the percentage of diseased tissue. This relationship was indicated by high correlation between disease severity rating and both infection cushion formation (r = 0.970) and lobate appressorium formation (r = 0.997). Therefore, the ability of the pathogen to form many infection structures on a given cultivar is a measure of the aggressiveness of the pathogen toward that cultivar.

The repression of the ability of the pathogens to form infection structures can be viewed as a defense mechanisms to disease. Since infection structures form only in response to the host and not in culture (4), it appears that a stimulus for their formation was being produced by the host. This phenomenon has been reported in other diseases caused by *R. solani* (6,21). An explanation of the differential response of the pathogen on resistant and susceptible cultivars could be the relative concentration of stimulating substance produced. Other possibilities, such as a stimulus produced by susceptible hosts and a stimulus-inhibitor complex produced by resistant hosts, also exist. The inability of *R. solani* to form infection cushions on hosts resistant to the pathogen was previously reported (6). The present study is the first account of this reaction occurring with *R. oryzae*.

No infection cushions were produced on resistant cultivars. Although lobate appressoria were formed on such cultivars and penetration occurred, subsequent invasion was slowed by the collapse of cells around the invading hyphae in the outer sheaths. The failure of the fungi to invade culms could be viewed as another defense mechanism of certain rice cultivars.

It was suggested by Flentje (5) that there are several stages during infection by *R. solani* when the process could be affected. These stages are; failure of the hyphae to attach to the plant surface; failure to form infection structures; failure of penetration pegs to penetrate; and failure of penetrating hyphae to continue invasion of the tissue due to a hypersensitive reaction or other limiting response. In the rice sheath blight and spot diseases, it is apparent that two of the conditions set forth by Flentje were in operation; viz, the failure to form infection structures and the failure of penetrating hyphae to continue invasion. The failure of hyphae to attach to the plant surface or the failure of the penetration pegs to penetrate was not observed.

These inabilities of the pathogens can now be studied in relation to breeding rice cultivars with inherent resistance to sheath blight and sheath spot. Results of the present study indicate that by breeding resistance to one of the diseases, resistance to the other is included. A preliminary study (11) indicated resistance in rice to infection cushion formation by *R. solani* was dominantly inherited in either a 3:1 or 13:3 ratio.

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