Stem rot of Rice in California

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ABSTRACT

A disease index based on the depth of penetration of *Magnaporthe salvinii* into the tiller was developed which correlated well with variations in inoculum levels, times of infection, cultivar susceptibility, and yield loss.

Under field conditions a 12% yield loss and a 12% reduction in the number of tillers/plant resulted when plants were inoculated with 500 viable sclerotia/plant (vs/p) during the period of stem elongation. Inoculations with equal or lower numbers of vs/p at mid-tillering or at the boot stage did not result in yield or tiller losses.

Six California cultivars varied in their degree of susceptibility to *Sclerotium oryzae*. 'Earlirose' was the most susceptible; 'Caloro', 'Calrose', 'CSM-3', and 'Kokohoe Rose' were moderately susceptible; and 'Colusa' was the least susceptible.

Yield losses up to 22% were measured under actual field conditions.

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In 1876, A. Cattaneo described *Sclerotium oryzae* Catt. as a pathogen causing stem rot of rice (*Oryza sativa* L.). *S. oryzae* has since been recorded in Ceylon (16), India, Burma, Madras (4, 17), The Philippines (18), Japan (11), North Carolina (9), Arkansas (22), and Louisiana (19). Tullis (20) reported that in 1932 *S. oryzae* was found on only one plant at the Biggs Rice Research Station in California; however, by 1933 it was found on six different cultivars in several commercial fields. He postulated that *S. oryzae* had been introduced into California from Arkansas on Early Prolific rice seed in 1931. Since its introduction, *S. oryzae* has spread throughout the northern rice-growing areas of California and is now causing increased concern among growers (7, 21). Losses caused by stem rot are due to increased tillering (4, 11), unfilled panicles (19), chalky grain (6), and increased lodging (10, 19). Although no actual loss measurements are available, estimates as high as 75% have been reported in Arkansas (6) and areas of the Punjab (15) and annual losses ranging from 5-10% throughout the rest of the rice growing world.

The present report deals with stem rot as it occurs in California; the organisms involved, a method to measure the relationship of symptom expression and yield loss, the effect and relationships of inoculum levels and time of infection, the role of conidia and ascospores in the disease cycle, and the relative susceptibility of California cultivars.

MATERIALS AND METHODS.–*Source of isolates and maintenance of cultures.*–All isolates were obtained from infected rice stems taken from Butte, Glenn, Sutter, and Colusa counties, California. The method of single spore isolation and maintenance of cultures have been described previously (7, 8).

Rice culture.–Plants for greenhouse experiments were grown in dried adobe-clay soil from the California Cooperative Rice Research Station (CCRRS) Biggs, California. The soil was screened (1.27 cm mesh screen), placed in 18.8-kg cans, moistened, autoclaved for 8 hr at 121 C, and aerated for 3 days before use. Soil analysis showed that sufficient phosphorous and potassium were present; however, 98 kg/ha of N was applied as $(NH_4)_2SO_4$. Five kg sterilized soil was placed in a nonperforated plastic bucket, distilled water was added to a water depth of 2.5 cm above the soil surface, and maintained at this level until the plants were 15 cm tall at which time the water level was raised to 10 cm. All seed was supplied by the CCRRS and the names and codes correspond to their cataloging system. Seeds were soaked 24 hr in a 1% solution of household bleach (5.25% sodium hypochlorite), and rinsed in distilled water. Fifteen seeds were then sown directly into the water onto the soil surface. The plants were thinned to six plants/pot 14 days after planting. The greenhouse was maintained at 27 to 36 C during the day and 24 to 27 C at night. The day-length was maintained at 14 hr with fluorescent light. Under these conditions all cultivars matured at their appropriate times.

Preparation of inoculum and inoculation of plants.–The method of sclerotial production, viability determination, and quantitative recovery from naturally infested soil has been published previously (8). Plants were inoculated 21 days after planting with 200 viable sclerotia/plant (vs/p), unless otherwise specified, by spreading a weighed amount of inoculum on the water surface of each pot. Sclerotia thus applied floated on the surface of the water and infected the plants upon contact. Where indicated, plants were wounded prior to inoculation by piercing each tiller with a blunt needle at the water line (14).

Disease ratings.–The disease index used in this study is a modification of Cralley's (6) system of disease measurement. Healthy and infected tillers were divided into five categories based on the amount of disease as follows: (i) healthy, no symptoms (Fig. 1H). Water stains on old and dried leaf sheaths could be distinguished from infected tissue by the presence of sclerotia in the latter (Fig. 1b); (ii) lightly infected with symptoms and sclerotia on the outer leaf

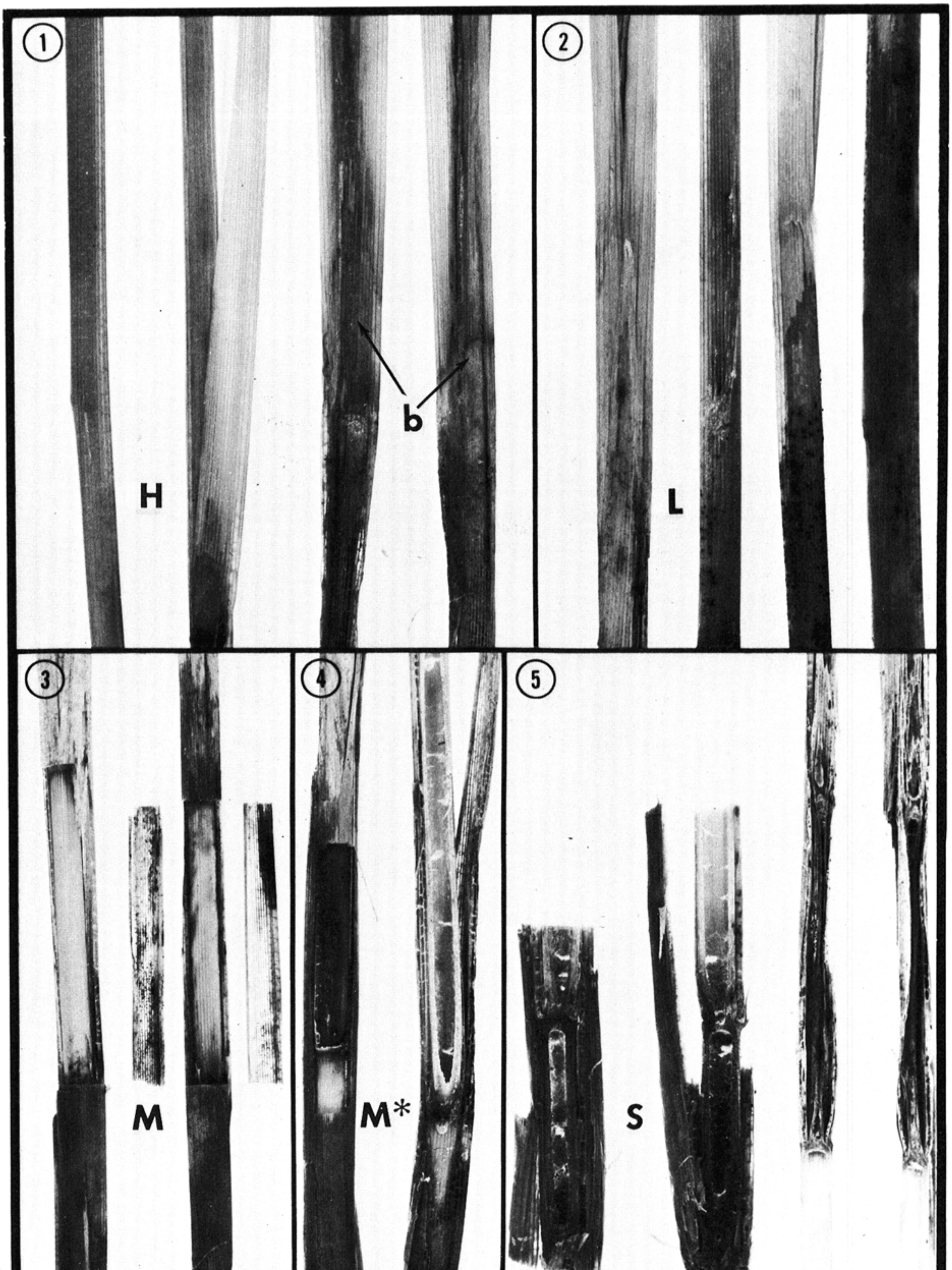

Fig. 1-5. 1) Healthy rice stems (H). Note the water stains on Fig. 1b which can be easily mistaken for infections. 2) Lightly infected rice stems showing symptoms on the outer leaf sheaths only (L). 3) Mildly infected rice stems showing discoloration of and sclerotia in the inner leaf sheaths, culm healthy (M). 4) Moderately infected rice stems, slight to moderate discoloration of the culm, interior of the culm healthy (M*). 5) Severely infected rice stems, culms infected internally, either collapsed or not (S).

sheaths only (Fig. 2); (iii) mildly infected with discoloration of and sclerotia in the inner leaf sheaths, culm green and healthy (Fig. 3); (iv) moderately infected, slight to mild discoloration of the culm, interior of the culm healthy (Fig. 4); (v) severely infected, culms infected internally, either collapsed or not (Fig. 5).

Each category was weighted and the disease index (DI) calculated as follows:

$$DI = \frac{1(H^n) + 2(L^n) + 3(M^n) + 4(M^{*n}) + 5(S^n)}{\text{Total number of tillers examined}}$$

where: H^n = number of healthy tillers, L^n = number of lightly infected tillers, M^n = number of mildly infected tillers, M^{*n} = number of moderately infected tillers, and S^n = number of severely infected tillers. Therefore, a DI of 1.00 represents all healthy tillers, and a DI of 5.00 all severely infected tillers. The DI was recorded for all experiments when the rice grain was translucent and clear, or when the grain was approximately 20% moisture. Rice at this stage of development was considered mature in all experiments.

Inoculum rate and time of infection.—Experiments were located at the University of California Rice Research Facility, Davis (UCD) where rice had not been grown previously and no sclerotia of *S. oryzae* could be detected. Continuous, nonperforated, aluminum rings, 30 cm deep, and 90 cm in diam were placed in the field and pressed 7.5 cm into the soil. The field was then flooded with 10 cm of water which diffused through the soil and into the rings. Three-week-old Earlirose seedlings were transplanted into each ringed area to give 160 plants/m^2. Six replicates, each with 0, 5, 50, and 500 vs/p, were inoculated at mid-tillering, internode elongation, and boot stage. Rice in the rings was hand-harvested at 146 days, and the disease index, total number of tillers, and yield were recorded for each ring.

Cultivar susceptibility.—Field tests were carried out as follows. Rings made of 0.64-cm mesh wire screen, 90-cm in diam, and 30-cm deep were placed in the fields before flooding in a randomized complete block design replicated four times. The rings were covered with plastic sheets to exclude all extraneous seed while the fields were being sown. After the fields had been sown the plastic sheets were removed and each ringed area seeded with the respective cultivar. Immediately after harvest, soil samples were taken from each trial. One 40-g sample was taken with a soil tube from each of the four corner areas and one from each of the four center areas. Samples thus taken from each of the three field trials indicated that Field 1 had 412 vs/p; Field 2, 38 vs/p; and Field 3, 10 vs/p. Although these figures included some sclerotia produced on the current crop, they showed that the inoculum levels of the three fields varied as desired and represented three distinct inoculum levels. Since there are reports that early cultivars escape damage due to stem rot because they are harvested before the disease becomes severe (3), each was harvested as it matured and the disease index was recorded.

Yield losses under field conditions.—To assess and correlate yield losses due to stem rot with the disease index, a 0.8 ha field trial was established at UCD on soil not previously planted to rice and free of any detectable sclerotia of *S. oryzae*. The experiment was divided into four 0.2-ha plots. They were dry-seeded with Earlirose seed, and flooded. The plots were maintained under standard cultural practices throughout the season. Two 0.2-ha plots were each inoculated 6 weeks after planting by hand-broadcasting 15 kg of the infected rice:rice hull mixture (8) over the surface of the water. Field observations had indicated that inoculum, on the surface of the water, was carried by the water flow to the lowest field basin, resulting in increased disease severity in that area. Based on these observations, the experiment was designed so that water free of *S. oryzae* flowed through the noninoculated plots and into the inoculated plots. The disease index was read for each plot in the following manner: Each plot was divided into two subplots beginning at the lowest plot (lowest water level) and going towards the highest plot. Immediately before harvest, three 0.09-m^2 samples (approximately 60-80 tillers) were cut from each subplot and the disease index was calculated. Each subplot was harvested with a John Deere 105 harvester, moisture samples were taken, and yields recorded.

RESULTS.—*Sclerotium oryzae* was the stem rot fungus isolated most frequently from rice stems and soil. Initial symptoms were small, black, elongated lesions which appeared at or below the water line 40 to 60 days after sowing (Fig. 2). As the season progressed the black diffuse lesions increased in size and often coalesced to encompass the entire outer leaf sheath (Fig. 3) and the lower internodes of the culm (Fig. 4). In severe infections, the culm was killed and abundant sclerotia and mycelia were produced inside the blackened lumen of the culm (Fig. 5).

The imperfect stage of *S. oryzae*, *Vakrabeeja sigmoidea*, occurred on the dead outer leaf sheaths just above the water line approximately 60 days after the crop was planted. The perfect stage, *Magnaporthe salvinii* (Catt.) Krause & Webster (= *Leptosphaeria salvinii* Catt.) (7), appeared in the dead infected outer leaf sheaths at approximately 100 days after planting.

Inoculum rates and time of infection.—The effect of inoculum rates was determined so that a standardized method could be used in all subsequent experiments. Since previous workers (13, 19) believed that wounding increased disease severity, preliminary greenhouse treatments included inoculum rates of 0, 5, 10, 50, 100, and 200 viable sclerotia/wounded and nonwounded plants. Puncturing the stems did not enhance disease severity (Table 1). Inoculum rates of 10, 50, 100, and 200 vs/p did not differ significantly in indexing disease severity, but five vs/p produced significantly less disease than the higher inoculum rates. Based on this evidence, an inoculum rate of 200 vs/p was adopted as a standard inoculum rate for all additional greenhouse experiments.

Subsequent experiments were carried out to

TABLE 1. The effect of different rates of viable sclerotia of *Sclerotium oryzae* per plant on stem rot severity on the rice cultivar Caloro under greenhouse conditions[a]

Inoculum rate[c]	1969[b] Disease index		1970[b] Disease index	
	Wounded	Nonwounded	Wounded	Nonwounded
0	1.00 d[d]	1.00 d	1.00 d	1.00 d
5	3.10 c	2.64 c	2.65 bc	3.35 ab
10	3.91 ab	3.17 c	3.34 ab	3.78 a
50	4.24 ab	4.16 ab	4.09 a	3.17 ab
100	4.28 a	4.24 ab	4.22 a	4.08 a
200	4.36 a	4.25 ab	4.25 a	3.97 a

[a] A randomized complete block design replicated five times.
[b] Each experiment analyzed separately.
[c] Viable sclerotia per plant.
[d] All disease index rating followed by the same letter do not differ significantly at the 1% level.

determine what inoculum rates were necessary for disease production under field conditions. Since previous reports (3, 12) stated that stem rot was more evident on plants nearing maturity, the effect of the time of infection on disease occurrence, severity, and yield loss was tested. Significantly more disease was caused by 50 and 500 vs/p than 5 vs/p which did not differ from the control. Therefore, under field conditions a rate greater than 5 vs/p is necessary for significant disease production.

A direct relationship also occurred between inoculum rates and time of infection (Table 2). Treatments inoculated at mid-tillering and at the boot stage with 5, 50, and 500 vs/p resulted in no yield loss or reduction in tillers/plant when compared to controls; whereas, a 12% yield loss and a 12% reduction in tillers/plant resulted when the plants were inoculated at internode elongation with 500 vs/p. Inoculation at internode elongation with 5 or 50 vs/p did not result in significant losses. Therefore, yields are reduced when a high inoculum rate is present at the critical period of susceptibility; i.e., between late-tillering and completed stem elongation.

Cultivar susceptibility.—The relative susceptibility of 'Earlirose', 'Calrose', 'Kokohoe Rose', 'Colusa', and 'Caloro' to *S. oryzae* was tested. In 1969 Colusa, Calrose, Caloro, and CSM-3 (a new selection from CCRRS) were tested in the greenhouse. All cultivars were equally susceptible and wounding had no effect. The same experiment was repeated in 1970 with the cultivars Earlirose, Colusa, Calrose, Caloro, Kokohoe Rose, and CSM-3. Earlirose was the most susceptible; Kokohoe Rose, Caloro, and CSM-3 moderately susceptible; and Colusa the least susceptible (Table 3).

The same cultivars were evaluated in 1970 in three naturally infested fields in Butte County. Based on our data that disease severity depended upon inoculum rates, three fields were chosen with differing histories of stem rot. Plants from Field 1 had been severely damaged by stem rot for the previous 3 years; Field 2 had a history of moderate stem rot for the last 3 years; and Field 3 had exhibited light to sporadic disease symptoms during the same period.

Under high inoculum levels Earlirose was the most susceptible, Colusa the least susceptible, and the other cultivars moderately susceptible. Under medium inoculum levels Earlirose was again the most susceptible; all other cultivars were moderately susceptible (Table 3).

Yield losses under field conditions.—The data in Table 4 show that inoculum is carried in the direction of water flow as indicated by the increased disease severity (DI) and yield losses in the lower subplots 1, 2, 3, and 4. A DI of 2.05 yielded 1,235 kg/ha, a loss of 22% as compared to the noninoculated check (subplot 8) which had a DI of 1.01 and a yield of 1,621 kg/ha. The average DI of the inoculated subplots was 1.62 resulting in an average yield of 1,305 kg/ha, and an 18% yield loss, whereas the noninoculated checks had an average DI of 1.08 and a

TABLE 2. The effect of inoculum rate and time of infection by *Sclerotium oryzae* on disease development and yield loss as determined under field conditions on the rice cultivar Earlirose[a]

Time of infection	Inoculum rate[b]	Disease index	Average yield g/ring	Tillers per plant
	0	1.00 a[d]	1,009 NS[c]	4.1 NS
Mid-	5	1.20 a	1,039 NS	4.4 NS
tillering	50	2.24 c	1,005 NS	4.1 NS
	500	2.79 d	995 NS	4.3 NS
	0	1.00 a	1,169 a	4.1 a
Internode	5	1.10 a	1,150 a	3.8 a
elongation	50	2.19 c	1,136 a	3.9 a
	500	3.18 e	1,035 b	3.6 b
	0	1.00 a	1,110 NS	3.8 NS
Boot	5	1.15 a	1,186 NS	4.0 NS
stage	50	1.60 b	1,130 NS	3.8 NS
	500	2.23 c	1,166 NS	4.0 NS

[a] A randomized complete block design replicated six times.
[b] Viable sclerotia/plant.
[c] NS = not significantly different.
[d] All figures in a given measurement followed by the same letter do not differ significantly at the 5% level.

TABLE 3. Susceptibility of six rice cultivars to *Sclerotium oryzae* as tested in three different fields each with different inoculum levels[a]

Cultivar	Field 1[b] disease index	Field 2[b] disease index	Field 3[b] disease index	Greenhouse 1970 disease index nonwounded
Earlirose[a]	4.20 a[c]	3.69 a	1.44 a	4.75 a
Caloro	3.89 b	2.95 b	1.19 a	4.12 b
Kokohoe Rose	3.70 bc	3.01 b	1.38 a	4.30 b
CSM-3	3.58 c	2.86 b	1.28 a	4.14 b
Calrose	3.53 c	2.80 b	1.21 a	4.05 b
Colusa	3.15 d	2.83 b	1.16 a	3.60 c

[a] A randomized complete design replicated four times.

[b] Field 1, 412 vs/p; Field 2, 38 vs/p; Field 3, 10 vs/p.

[c] All figures in a given measurement followed by the same letter do not differ significantly at the 5% level in Fields 1, 2, and 3; greenhouse data at the 1% level.

yield of 1,572 kg/ha. Therefore, under field conditions and the inoculum rates used in this experiment, yields corresponded to the DI.

DISCUSSION.–In order to make a comprehensive study of rice stem rot, and to develop possible control measures, a method was required to effectively measure and correlate disease occurrence and severity with yield loss under various conditions. The method developed by the International Rice Research Institute (1, 2), based on the length of lesion on the leaf sheath or culm, did not relate to disease severity and losses under California conditions. We also found that the method proposed by Chauhan et al. (5), based on the percent of infected tillers and the panicle weights of diseased versus healthy tillers, did not relate yield loss to disease severity in California. Under our conditions the secondary tillers, which ordinarily produce smaller panicles than the main tillers, are more severely affected by stem rot. Therefore, a comparison of panicle weights of healthy (main) tillers to panicle weights of diseased (secondary) tillers is not justified. Cralley's method (6) as here modified, correlated well with disease severity and yield loss in both greenhouse and field tests when the DI was read at maturity. This modified disease index method made possible a reliable evaluation of various factors affecting stem rot.

TABLE 4. The correlation of disease index on the rice cultivar Earlirose to yield loss as influenced by movement of sclerotia of *Sclerotium oryzae* under field conditions

Plot	Subplot	Treatment	Disease index[b]	Yield kg/ha	% Loss per acre[c]
1	1[a]	inoculated	2.05	1,235	22
	2	inoculated	1.51	1,327	16
2	3	inoculated	1.49	1,340	15
	4	inoculated	1.41	1,319	17
3	5	noninoculated	1.15	1,467	7
	6	noninoculated	1.11	1,649	0
4	7	noninoculated	1.03	1,568	1
	8	noninoculated	1.01	1,621	0

[a] Wind direction and water flow was from subplot 8 to subplot 1.

[b] Based on three samples of 60-80 tillers from each subplot.

[c] As % of average yield of noninoculated plots.

Earlirose, an early maturing medium grain cultivar, was consistently the most susceptible; Colusa, an early maturing short grain cultivar, was the least susceptible. All of the late maturing cultivars were moderately susceptible regardless of the grain length. Atkins (3) reported that early maturing cultivars escape damage if sown early and harvested early because stem rot is a late-season disease. However, our findings support the work of Cralley (6) who found that early, short grain cultivars were generally more resistant and that the resistance of these cultivars was a varietal characteristic and not directly associated with the time of maturity.

Disease occurrence and severity is also affected by inoculum rates and time of infection. Field inoculum rates must be at least 30-50 viable sclerotia/plant (Table 2), and this inoculum must be present between late-tillering and internode elongation to result in significant yield losses. Under these conditions, mild infections resulted in 18 to 22% yield losses. Conidia and ascospores of *M. salvinii* which are normally produced after this critical period probably do not cause infections which result in yield losses during the current season. Thus, the possible role of secondary inoculum would be to infect additional plants and thus produce more sclerotia to serve as primary inoculum the following year. Such infections are evident in the late season and would be placed in the light or mild categories of the DI.

Field observations indicate that any circumstances that reduce the vigor of the rice plant increase its susceptibility to *S. oryzae*. Severe disease has been observed in fields where damage by wind or chemicals has occurred resulting in an abundance of dead leaf tissue which probably served as entrance courts or a food base for the fungus. Similar conditions have also been observed where stand density is heavy resulting in shading and death of the lower leaves. Thus, although direct wounding did not increase disease severity in our experiments, the significance of dead or weakened plant tissue at the water level should not be overlooked.

Based on the results of this study, investigations on possible control measures have been initiated. Emphasis is being given to the effect of various cultural practices on inoculum levels in the soil, predisposition to infection, and a search for resistant or tolerant selections for use in breeding improved cultivars.

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