Evaluation of Virulence in Isolates of Sclerotium oryzae

S. A. Ferreira and R. K. Webster

Department of Plant Pathology, University of California, Davis 95616. Current address of senior author is Department of Botany and Plant Pathology, Colorado State University, Ft. Collins 80523.

This study was supported by the California State Rice Research Board. The authors thank J. N. Rutger and J. M. Duniway for helpful suggestions and for reviewing the manuscript.

Accepted for publication 29 March 1976.

ABSTRACT

FERREIRA, S. A., and R. K. WEBSTER. 1976. Evaluation of virulence in isolates of Sclerotium oryzae. Phytopathology 66:1151-1154.

A test to measure the disease reaction of individual *Sclerotium oryzae* isolate-host combinations was developed by estimating the ability of isolates to incite lesions or kill 25-day-old Colusa rice seedlings. Since the ability of isolates to incite lesions was correlated with the ability to kill seedlings, virulence of individual isolates can be determined by measuring their ability to incite lesions. When Colusa and Earlirose rice were compared by the seedling test, the initiation of lesions was delayed more on Colusa, indicating that Colusa is more resistant to *S. oryzae* than Earlirose. Therefore, the seedling test can be used to evaluate rice cultivars for stem rot resistance as well as virulence in *S. oryzae*.

Additional key words: stem rot of rice.

Stem rot of rice (*Oryza sativum* L.), which is caused by *Sclerotium oryzae* Catt., is first apparent in the field as small dark lesions on the leaf sheaths at the water level. Disease progression is characterized by the death and sloughing of the infected sheaths followed by penetration of the entire culm. When the culm is infected, both grain quality and panicle size are reduced. When infection occurs early in the season, tillers either are killed or fail to produce panicles. Additional reduction in yield results from increased lodging of infected plants.

Few studies exist concerning the virulence of *S. oryzae* and its potential for variation within a population of the pathogen. This stems mostly from the lack of reliable procedures for evaluating the virulence of different isolates. Ou (1, 2, 6) reported that isolates of *S. oryzae* differed in their ability to cause lesions on excised stems of rice. Based on lesion length, he characterized the relative resistance of approximately 600 rice cultivars on a scale of 0 to 9. He did not, however, quantify the existing or potential variation in virulence in *S. oryzae*. Consequently, studies were initiated to determine whether Ou's (1, 2, 6) or another method could be employed to evaluate the virulence of different isolates of *S. oryzae* in California. Such a method would be invaluable in studying pathogen variation and screening for host resistance where reactions on large numbers of individuals must be determined.

MATERIALS AND METHODS

Isolates of Sclerotium oryzae.—Single conidial isolates of *S. oryzae* were obtained which differed in their capacity to cause disease on cultivar Calrose rice plants inoculated with sclerotia. The ability to cause disease was determined in greenhouse trials; plants were rated at maturity for disease using the index of Krause and Webster (5). Additional single conidial isolates were obtained from single, newly formed lesions on rice from four widely separated fields in Butte County, California. All isolates were maintained on Difco cornmeal agar (CMA).

Sclerotium production.—Sclerotia, used as inoculum in most experiments, were produced as follows. Rice hulls and unmilled rice were mixed in a 2:1 ratio and wetted with a solution consisting of 4 g $CaCO_3$, 1 g $CaNO_3$, 0.25 g $MgSO_4$, 0.25 g KCl, 0.25 g KH_2PO_4, 1 g sucrose, and 1 liter distilled water. The wet hull-rice mixture was placed in canning jars, the lids of which had been punctured and fitted with sponge plugs. After being autoclaved for 90 minutes, the jars were cooled and agar plugs of the desired isolates were added to the jars. After 6 weeks of incubation at room temperature, the rice-hull cultures were dried for 48 hours on the laboratory bench. Sclerotia were separated from the incubation mixture by screening the dry material through two layers of cheesecloth. Sclerotia were stored at 4 C until used.

Seedling inoculations.—Rice seedlings of the cultivars Colusa, Calrose, and Earlirose were used in most of the experiments. Seedlings were grown in the greenhouse under a 16-hour light period in pots with pasteurized Yolo loam soil topped with 1-2 cm of sand. Seedlings were uprooted, washed, and placed in 17 ml of Hoagland's solution in test tubes (25 × 200 mm). The Hoagland's solution used was that described previously for rice culture (3, 4). Leaves of the seedlings were trimmed so that none protruded from the tubes. About 4 mg of sclerotia measured with a standard small scoop were floated on the Hoagland's solution in which the seedling had been placed. Surface tension caused sclerotia to "adhere" to the stem, simulating the conditions in a flooded rice field. First symptoms of infection, measurement of lesion dimensions, and the number of killed seedlings were noted daily. The expression of disease was termed disease reaction and the disease

Copyright © 1976 The American Phytopathological Society, 3340 Pilot Knob Road, St. Paul, MN 55121. All rights reserved.

reaction of particular isolate-host combinations was considered to be a measure of the virulence of the isolate. Variation in disease reaction could be a function of variation in host resistance, pathogen virulence, or experimental conditions.

Greenhouse trials.—For greenhouse inoculations, individual Colusa seedlings were transplanted to 22-cm diameter pots half-filled with pasteurized Yolo loam soil and maintained with 6-10 cm of standing distilled water. Six-week-old plants were inoculated by placing 150 mg of sclerotia on the water, and were rated for disease by the method of Krause and Webster (5) at 18 weeks of age.

RESULTS AND DISCUSSION

To determine if age of plants (days from seeding) and/or inoculum level influenced results of the seedling inoculations, these factors were studied. Groups of 10 Colusa rice seedlings 7, 14, 21, and 28 days old were inoculated with 4 mg of sclerotia from each of two isolates and observed for number of infected seedlings vs. number of killed seedlings 1, 3, 6, and 7 days after inoculation. Lesions first were observed on 7- and 14-day-old seedlings, 3 days after inoculation with both isolates (D-51, and C-6) that were tested. Only isolate C-6 caused lesions after 3 days on 21- and 28-day-old plants. Both isolates caused lesions on 7-, 14-, and 28-day-old plants 6 days after inoculation. Thus, a difference of time required for production of lesions between isolates was observed on both 21- and 28-day-old plants. Consequently, 25-day-old plants were used in subsequent tests.

The results of inoculating 25-day-old Colusa rice seedlings separately with three isolates of *S. oryzae* at three inoculum levels are given in Table 1. When isolates were evaluated for disease reaction in the tube test, the inoculum levels tested gave near equal results with a slight advantage seen in the higher levels. Therefore, 5 mg of sclerotia was used as the inoculum level in subsequent tests. Repeated tests gave similar results.

TABLE 1. The effect of inoculum level on the disease reaction of 25-day-old Colusa rice seedlings to three isolates of *Sclerotium oryzae*

Days after inoculation	Disease reaction of Colusa rice seedlings inoculated with:								
	2-mg of sclerotia			4-mg of sclerotia			10-mg of sclerotia		
	Isolate			Isolate			Isolate		
	D-30	AV-17	AV-13	D-30	AV-17	AV-13	D-30	AV-17	AV-13
3	7[a]-0[b]	0-0	0-0	8-0	2-0	0-0	10-0	1-0	0-0
5	10-4	7-0	4-0	10-5	8-0	6-0	10-0	8-0	6-0
7	10-10	8-0	9-0	10-10	10-0	9-0	10-10	10-10	8-0
9	. . .	10-0	10-0	. . .	10-2	10-0	. . .	10-2	9-0
16	. . .	10-4	10-1	. . .	10-6	10-4	. . .	10-6	10-4

[a]The first of the pair of numbers represents the number of infected seedlings of 10 that were inoculated.
[b]The second number represents the number of killed seedlings of 10 that were inoculated.

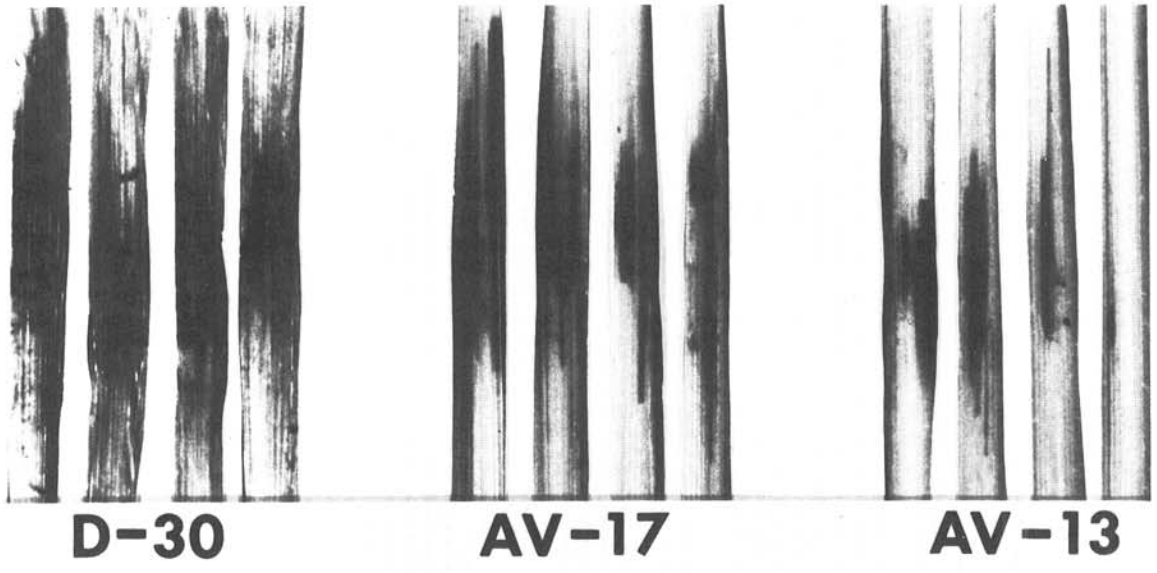

Fig. 1. The disease reaction 7 days after inoculation with three isolates (D-30, AV-17, and AV-13) of *Sclerotium oryzae* on 25-day-old Colusa rice seedlings. Four seedlings were each inoculated with 10 mg of sclerotia of each isolate and representative lesions are shown.

Disease reaction as determined by seedling inoculation tests.—The results of inoculating 25-day-old Colusa, Calrose, and Earlirose seedlings with different isolates of *S. oryzae* showed that isolates varied in ability to initiate lesions or to kill rice seedlings (Fig. 1). Isolates that had higher disease indices when inoculated to rice plants in the greenhouse tended to initiate lesions sooner and to kill more seedlings in the seedling tests. In some cases, isolates caused severe reactions on one cultivar but not on another, indicating that the differential virulence observed among isolates was not due to differences in the ability of sclerotia to germinate.

Results were similar when 20-cm pieces of stem tissue from 7-week-old Colusa and Calrose plants were inoculated following the method described by Ou (1, 2, 6). It was more difficult, however, to distinguish the isolates of *S. oryzae* on the basis of their virulence on stem pieces because the disease reaction was more uniform, which masked the differences between isolates. The ability to determine the extent of variation in virulence within a large sample of isolates by use of the seedling test was examined by inoculating Colusa seedlings with 69 separate isolates of *S. oryzae*. Ten seedlings were used for each isolate. Figures 2 and 3 show the results of tests in which the time for half of the 10 seedlings to show symptoms or be killed was used as a quantitative measure of disease reaction. As seen in Fig. 2 and 3, the majority of the 69 isolates were intermediate in their ability to initiate lesions or to kill seedlings and the virulences of the isolates were somewhat normally distributed.

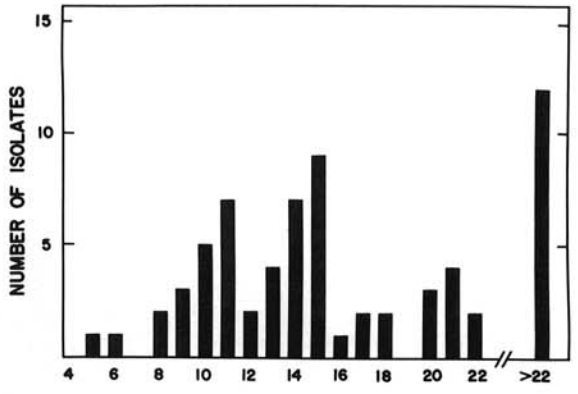

Fig. 2. The distribution among 69 isolates of *Sclerotium oryzae* of the disease reaction on 25-day-old Colusa rice seedlings. Disease reaction was measured by the time for half of 10 inoculated seedlings to show symptoms.

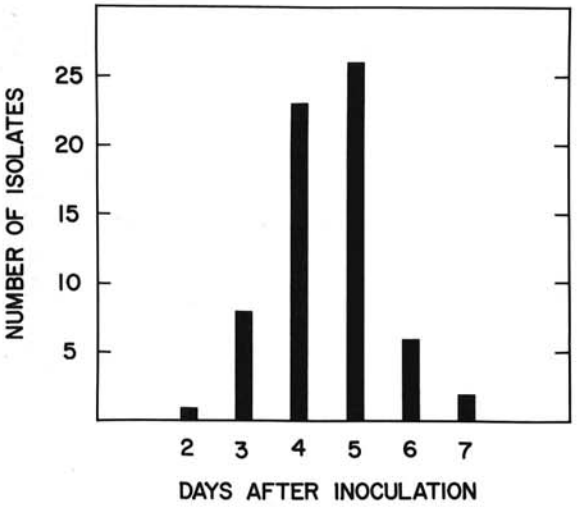

Fig. 3. The distribution among 69 isolates of *Sclerotium oryzae* of the disease reaction on 25-day-old Colusa rice seedlings. Disease reaction was measured by the time for half of 10 inoculated seedlings to be killed.

Lesion length or width was highly variable among the seedlings inoculated with a single isolate. However, seedlings with broad lesions tended to be more severely affected and were killed more quickly. Variation in lesion length and width may result from natural variation among seedlings or the manner in which sclerotia adhered to stems when seedlings were inoculated. Some lesions were initiated from relatively few sclerotia whereas others resulted from accumulations of many sclerotia at the stem. We concluded that any method based on lesion length to estimate the virulence of *S. oryzae* is subject to variation that would make it difficult to quantify the virulence of individual isolates. This raises some doubt about the applicability of Ou's (1, 2, 6) stem tissue method to evaluate the virulence of *S. oryzae*.

The relationship between the ability to initiate lesions and to kill seedlings among siolates of *S. oryzae* is shown by correlation and linear regression analysis in Fig. 4. These two parameters measured by the seedling test are directly related to each other. Therefore, one needs only to determine lesion initiation to estimate the disease reaction of a particular isolate-host combination.

Comparison of seedling and mature plant disease reactions.—If virulence was the character measured with the previous seedling inoculations, evaluation of isolates by the seedling test might be expected to be similar to that by the disease index of Krause and Webster (5) on mature plants. To determine if such is the case, six isolates which

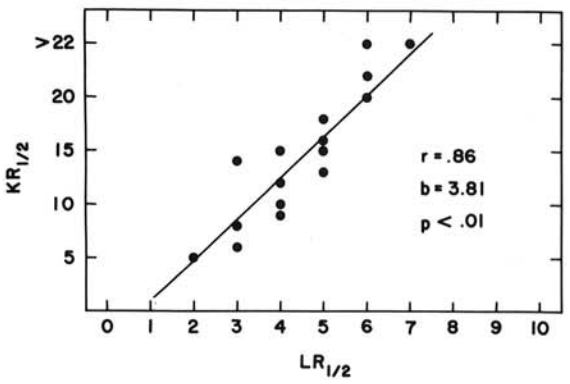

Fig. 4. Correlation and regression of the ability to initiate lesions (LR 1/2) and the ability to kill seedlings (KR 1/2) for 16 isolates of *Sclerotium oryzae*. LR 1/2 = time in days for half of 10 inoculated 25-day-old Colusa rice seedlings to show symptoms. KR 1/2 = time in days for half of 10 inoculated 25-day-old Colusa rice seedlings to be killed.

TABLE 2. Comparison of disease reaction of six isolates of *Sclerotium oryzae* on Colusa rice seedlings with disease index determined on mature plants in greenhouse trials

Isolate	LR 1/2[y]	KR 1/2[w]	Disease index[x]
D-30	2	5	3.4 a
G-22	3	9	2.6 b
D-43	3	7	2.7 b
B-10	5	16	2.9 ab
D-19	6	22	2.8 ab
D-17	7	22+	2.5 b

[x]LR 1/2 = Time in days for half of 10 inoculated seedlings to show symptoms.

[y]KR 1/2 = Time in days for half of 10 inoculated seedlings to be killed.

[z]Values followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

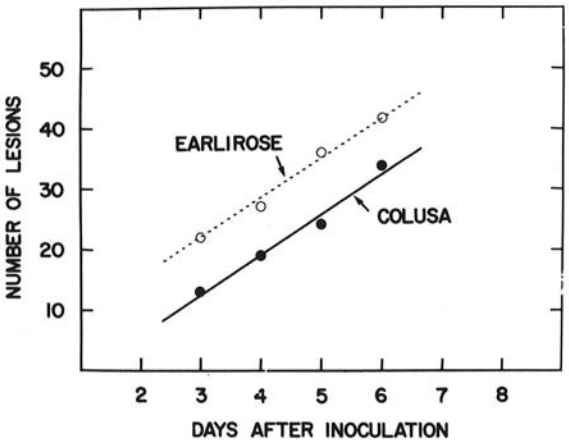

Fig. 5. Lesion initiation on Colusa rice and Earlirose rice seedlings. Twenty isolates of *Sclerotium oryzae* were inoculated to 60 seedlings of each cultivar. The number of lesions which were initiated on 25-day-old rice seedlings is plotted against time.

differed in virulence in the seedling test were selected and compared for mature plant disease reaction. Sclerotia from individual isolates were inoculated to each of seven Colusa plants when they were 6 weeks old. Plants were harvested after 18 weeks, and rated for disease index (5).

The results (Table 2) show that one of the isolates, D-30, differed from the other isolates in ability to cause disease as measured by the disease index; in addition, this isolate caused lesions and killed seedlings more rapidly than any other isolate studied with the seedling test. It appears that the seedling test does measure the virulence of *S. oryzae,* particularly in isolates with extreme abilities to cause disease (e.g., D-30). The differences among isolates of the pathogen made evident by the seedling test were not as apparent on mature plants. This may have been the case because the experiment was conducted during the winter, when because of lower temperatures, lower light intensity, and the short daylength, rice matured later and tended to tiller excessively. This extended period for maturity and tillering may have masked some of the differences in virulence of isolates indicated by the seedling test.

Use of the seedling test to determine differences in disease reaction between rice cultivars.—To determine if the seedling test might be useful in rapidly screening for stem rot resistance in rice, two cultivars which were known to differ in susceptibility to *S. oryzae* in greenhouse trials, were compared. Sixty each of 25-day-old Colusa and Earlirose seedlings were inoculated with a mixture of sclerotia from 20 isolates. The onset of lesion formation was followed with time. The rate of lesion formation on the two cultivars was the same (Fig. 5), but the onset of lesion development was delayed about 1 day on the more resistant Colusa. The seedling test appears to differentiate degrees of rice susceptibility to stem rot.

The procedure of inoculating rice seedlings described here is considered reliable for determining relative differences in virulence among isolates of *S. oryzae* and differences in resistance between various genotypes of rice. Since the seedling test can measure the virulence of isolates, and because the varieties Colusa and Earlirose differed in susceptibility to stem rot as indicated by both field, greenhouse (5), and the seedling test, it could be invaluable in screening for stem rot resistance in a breeding program for rice improvement. This would be particularly true if stem rot resistance proves to be quantitatively inherited.

LITERATURE CITED

1. INTERNATIONAL RICE RESEARCH INSTITUTE. 1965. Pages 112-114 *in* Annual Report of the IRRI, Los Baños, The Philippines. 357 p.
2. INTERNATIONAL RICE RESEARCH INSTITUTE. 1966. Pages 89-90 *in* Annual Report of the IRRI, Los Baños, The Philippines. 302 p.
3. KEIM, R. 1972. The effects of nitrogen, phosphorus, and potassium on severity of stem rot disease of rice. M.S. Thesis. University of Calif., Davis. 63 p.
4. KEIM, R., and R. K. WEBSTER. 1974. Nitrogen fertilization and severity of stem rot of rice. Phytopathology 64:178-183.
5. KRAUSE, R. A., and R. K. WEBSTER. 1973. Stem rot of rice in California. Phytopathology 63:518-523.
6. OU, S. H. 1972. Rice diseases. Commonwealth Mycological Institute. Kew, Surrey, England. 368 p.