Screening Techniques for Stem Rot Resistance in Rice in California

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ABSTRACT

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Inoculum production and inoculation techniques are reported. Rating plants 35–42 days after 50% flowering in the field produced the highest stem rot disease severity scores and differentiation among cultivars previously known to vary in susceptibility and growth duration. A fertilizer rate of 0.21 g of nitrogen per pot (15.8 kg of N/ha) as 16-20-0 applied 21 days after seeding differentiated among cultivars nearly as well as higher nitrogen rates and minimized pest proliferation in the greenhouse. Correlations between field and greenhouse disease ratings ranged from $r^2 = 0.89$ to 0.94 (significant at $P = 0.03$ to 0.06) within years and from $r^2 = 0.36$ ($P = 0.40$) to 0.94 ($P = 0.03$) between years. Correlations of between-year greenhouse disease ratings ranged from $r^2 = 0.47$ ($P = 0.31$) to 1.00 and from $r^2 = 0.67$ ($P = 0.18$) to 0.97 ($P = 0.02$) for field disease ratings. Correlations of stem rot rating with number of sclerotia formed per tiller for all cultivars ranged from $r^2 = 0.51$ to 0.60 ($P < 0.001$).

Stem rot caused by *Magnaporthe salvinii* (Cattaneo) Krause & Webster (sclerotial state = *Sclerotium oryzae* Cattaneo) occurs worldwide and is a major disease of rice (*Oryza sativa* L.). Field losses have been estimated at as high as 80% (12). The disease is widespread in California (15), and yield reductions of 12–22% under inoculated conditions and 6–23% under natural conditions have been documented (8,11). Although a chemical capable of controlling stem rot in California has been reported (8), it was denied registration. Sclerotia are the primary overwintering structures of this fungus, and they initiate the phase of the disease primarily responsible for yield losses within a season (11). Burning fields to destroy sclerotia limits disease buildup, but resultant air pollution may restrict this practice (4). Because the half-life for sclerotia in soil is 1.9 yr, crop rotation or fallowing are generally ineffective as disease control measures (4). Cultivars differ in resistance to this disease, but other breeding programs for high stem rot resistance have apparently either been unsuccessful or not initiated (12). Recently, resistance was found in a wild species of rice in California, and a resistant germ plasm line was released (7,14). Breeding for disease resistance seems to be the best alternative for controlling this disease in California.

Studies on the effects of time of inoculation, rate of inoculum application (11), and field fertilizer rate (9,10) on stem rot have already been published. Correlations of stem rot rating with inoculum level, time of infection, cultivar susceptibility, yield loss, and overwintering sclerotia have also been reported (3,4,11). Other resistance screening parameters, including fertilizer rate in greenhouse tests, correlation of stem rot rating to sclerotium production, time of rating, and correlation of greenhouse and field stem rot ratings, were studied in this research.

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MATERIALS AND METHODS

Rice culture, inoculation, and rating. All research was conducted at the Rice Experiment Station, Biggs, California, on Stockton clay adobe soil. Standard cultural practices for rice production in California were followed (13), except as noted below. F_2 populations were seeded at 12 cm between positions (two seeds per position to avoid blank spots) with a precision planter in rows 12.2 m long and 0.3 m apart. Advanced generation rice lines were sown in 1.2-m rows spaced 0.3 m apart with a hand seeder. One-hundred-twelve kg/ha of 18-46-0 plus 700 kg/ha of ammonium sulfate were preplant incorporated (169.6 kg of N/ha), and 168 kg of ammonium sulfate per hectare (35.3 kg of N/ha) was top-dressed by airplane 55 days after seeding. A fertilizer spreader set to deliver 2 ml (about 0.5 g or 3.75×10^4 sclerotia) per meter of row at 59, 68, 64, and 67 days after seeding was used to inoculate plants in tests conducted in 1984–1987, respectively. Sclerotia spread out when they hit the water surface and contacted plants at the waterline. A hectare could be inoculated in 1.2 hr with this technique.

Plants were rated for stem rot severity on a scale of 0–10. Zero represented no infection; 1 = all but three leaf sheaths enclosing the culm at the water line (12.5 cm height on the plant) penetrated; 2 = all but two leaf sheaths penetrated; 3 = all but one leaf sheath penetrated; 4 = all leaf sheaths penetrated (usually leaf sheaths 5, 4, or 3, numbered from the flag leaf down); 5 = superficial mycelial growth on the culm; 6 = first visible discoloration of the culm; 7–10 = 25%, 50%, 75%, and 100% girdling of the culm, respectively. This scale was expanded compared to that reported by Krause and Webster (11) to specify which leaf sheath and how much of the culm was infected, thus allowing finer differentiation among cultivars being tested for disease resistance. Because research indicates that the extent of sclerotium formation depends on the plant part and/or the part position infected (J. Oster, *unpublished*), this new scale may also allow evaluation of the potential of cultivars for sclerotium production.

Inoculum production. Widemouth 2-L canning jars were two-thirds filled with a 2:1 (rough rice:rice hull) mixture. Water was added to cover the mixture by 1–2.5 cm. Jar mouths were covered with Whatman No. 54 filter paper, which was secured with standard canning bands. Jars were placed horizontally in an autoclave for 30 min at 121 C, shaken to distribute moisture, autoclaved for an additional 60 min, and shaken again. Each jar was inoculated with a 1-cm^2 plug of a 5- to 10-day-old culture of *S. oryzae* growing on potato-dextrose agar. After incubation at 20–25 C for 3 wk, the inoculum was placed on a forced-air dryer for 1 wk. It was then passed through a sample sheller to separate rice grains from hulls and sclerotia. To separate sclerotia from hulls and other debris, they were passed through a 2.18-mm pore seive and then a U.S. No. 18 screen with 0.99-mm openings. This method is modified from that of Krause and Webster (11). Twenty different single-spore isolates of the stem rot pathogen collected throughout Butte County, California, were used for inoculum production, including the highly virulent isolate D-30 reported by Ferreira and Webster (6). Single-spore isolates were obtained by surface-sterilizing sclerotia for 3 min in a 10% solution of

5.25% sodium hypochlorite with two drops of Liqui-nox (a biodegradable, phosphate-free liquid detergent) added per 100 ml. Sclerotia were then rinsed three times in sterile, deionized water, air dried, and plated onto 1.5% water agar amended with 100 ppm each of penicillin G and streptomycin sulfate. Conidia were produced on the sclerotia within a week. A fine needle was used to streak conidia onto water agar plates, and individual germinating spores were transferred to potato-dextrose agar. Cultures were prepared for long-term storage by growing each isolate in a mixture of moist soil and wheat bran, allowing the soil to completely dry, and placing cultures in a freezer at 0 C (5). Viability has been maintained for 6 yr with this technique.

Time of rating. The California rice cultivars M-101 (CI 9970), M-201 (CI 9980), S-201 (CI 9974), M-302 (CI 9976), L-202 (PI 483097), and M-7 (CI 9967) were used in 1984 and 1985. M-101 is very early; M-201, S-201, and L-202 are early; M-302 is intermediate; and M-7 is late-maturing. Historically, M-101 has been significantly more susceptible to stem rot than the other cultivars. M-302 has been more susceptible than M-201 (the most resistant cultivar) but not significantly different from the remaining cultivars. In both years, cultivars were seeded into furrows in rows 1.2 m long and spaced 0.3 m apart at the rate of 0.8 g of dry seed per meter of row. Treatments were replicated four times in a factorial design, with time of rating as the main plot and cultivars as subplots. Furrows were raked to cover the seed, fields were flooded and drained (flushed) twice to establish a stand, and then fields were flooded (about 21 days after seeding) for the rest of the season. Ratings of 45 tillers per subplot replication (1) were taken at flowering and 14, 28, and 42 days after flowering in 1984. In 1985, ratings were taken at 21, 35, and 42 days after flowering. Results were subjected to analysis of variance (2).

Stem rot rating and number of sclerotia produced per tiller. In 1986 and 1987, fields were preplant fertilized with 560 kg of ammonium sulfate per hectare (117.6 kg of N/ha) and flooded. Presoaked (24 hr submerged, 24 hr drained at 21–24 C) seed was hand-sown into the water with an aluminum trough with a slit in the bottom at the rate of 1 g of dry seed per 1.2-m row. Fields were topdressed with 150 kg of ammonium sulfate per hectare (33.6 kg of N/ha) 55 days after seeding. The cultivars M-101, M-201, S-201, L-202, and D-16 (PI 506229), a line with a high level of stem rot resistance (13), were arranged in the field in a factorial design replicated four times. Time of rating was the main plot, and cultivars were subplots. Results were subjected to analysis of variance. Plants were harvested by cutting two subsamples per row at 14, 35, 56, and 77 days after flowering in 1986. The samples were frozen until stem rot ratings and number of sclerotia per tiller could be evaluated. All sclerotia were counted in each leaf sheath and in the culm of each of 15 tillers per replication with the aid of a binocular microscope equipped with a grid mounted in one eyepiece. Culms were split open before counting. Sampling times in 1987 were 0, 21, 35, and 77 days after flowering.

Greenhouse screening. Nonperforated plastic flower pots 16 cm in diameter and 12.7 cm in depth were filled with 1 L of Stockton clay adobe soil, placed in plastic-lined troughs on benches, and watered to saturation. Seven equally spaced seeds were sown into each pot and covered with soil. Greenhouses were maintained between 27 and 36 C during the day and 21 and 24 C at night. Approximately 21 days after seeding, the bench was flooded to cover the pots, and each pot was fertilized with 0.21 g of nitrogen (1.3 g per pot or 98.8 kg/ha of 16-20-0). Flood level was maintained with a float valve. Approximately 35 days after seeding, each pot received 0.32 g of nitrogen (1.5 g per pot or 115.2 kg/ha of ammonium sulfate). Nitrogen totalled 0.53 g per pot or 40 kg/ha. Plants were inoculated 45 days after seeding by shaking sclerotia from 1-L widemouth canning jars with seven 4-mm holes punched in each lid. Each pot received about 1.25×10^4 sclerotia to ensure infection of every tiller. Disease ratings were taken 35–40 days after flowering.

Greenhouse fertilizer trials. Because nitrogen has a major effect on stem rot development (9,10), trials were conducted to determine optimal rates of nitrogen application for disease resistance screening in the greenhouse. M-101 (the most susceptible to stem rot of current California cultivars) and M-201 (the most resistant of current California cultivars) were used in these trials. Techniques were the same as for the greenhouse screening experiments, except three rates of nitrogen were applied at 21 days after seeding (0.21 g of N per pot as 16-20-0 alone or in combination with 0.32 or 0.64 g of N as ammonium sulfate). Nitrogen was applied as ammonium sulfate 35–38 days after seeding, and inoculation occurred at 45–48 days after seeding. Treatments were replicated five times. Trials were rated 35–40 days after flowering, and results were subjected to a factorial analysis of variance.

Correlation of greenhouse and field ratings over years. Trials were conducted in the greenhouse and in the field in 1984–1988 with the use of the practices described in the time-of-rating trial. M-101, M-201, L-202, and the resistant D-16 line were used. These rices represented a range of susceptibility to stem rot. The purpose of the experiments was to determine if greenhouse results reflected field ratings and whether ratings in each environment were consistent over time. Cultivars were arranged in a randomized complete block design and replicated six times, and 15 tillers were rated per cultivar replication. Results were analyzed by linear correlations.

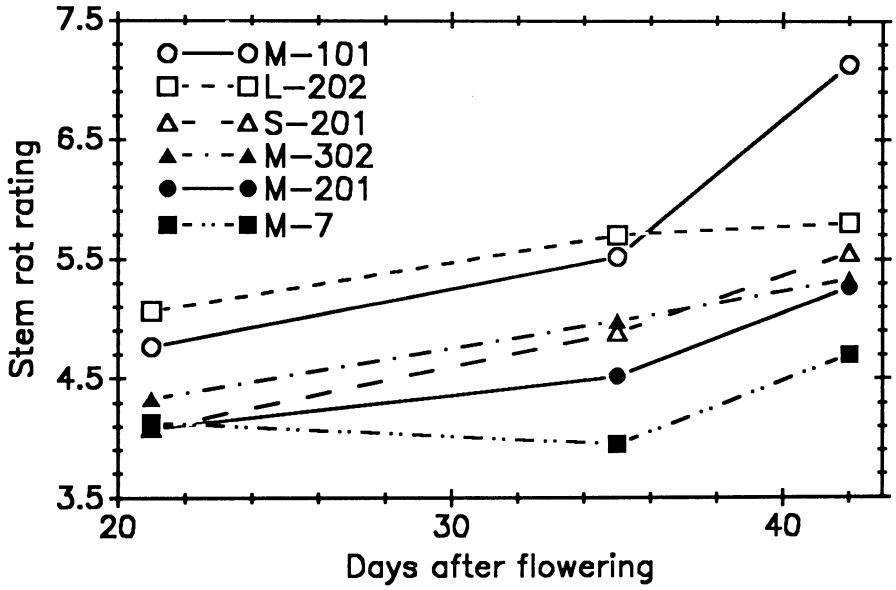

Fig. 1. Effect of time of rating on stem rot severity in 1985 for six rice cultivars (M-101, L-202, S-201, M-302, M-201, and M-7). LSD = 0.39 for time of rating, 0.30 for cultivar, and 0.67 for time of rating × cultivar interaction ($P = 0.05$). Ratings based on a 1–10 scale.

RESULTS

Time of rating. Disease severity and differences in disease rating among cultivars were greater in 1985 than in 1984, but disease ratings increased similarly over time in both years. Results are presented for 1985 (Fig. 1). Disease severity on most cultivars increased most rapidly between 35 and 42 days after flowering.

Differences among cultivar disease ratings were greatest at 42 days after flowering. This was reflected by significant cultivar × rating time interactions in both years (Fig. 1).

Stem rot rating and number of sclerotia produced per tiller. Correlations between stem rot rating and the number of sclerotia produced per tiller for all cultivars ranged from $r^2 = 0.51$ to 0.60 ($P < 0.001$) in 1986 and 1987. Only 1987 data are presented (Fig. 2). Individual cultivar correlations ranged from $r^2 = 0.89$ to 0.995 ($P < 0.001$) in 1986, except for D-16 ($r^2 = 0.33$, $P = 0.04$) and from $r^2 = 0.52$ to 0.77 ($P < 0.001$), in 1987. Few sclerotia had formed by flowering time. As in the time-of-rating study, the cultivars varied in disease rating, and disease severity on many cultivars increased sharply between 21 and 35 days after flowering. Cultivar differences in stem rot rating were greatest 35 days after flowering. A rapidly increasing stem rot score with relatively minor increases in sclerotia per tiller in most cultivars may indicate that accelerating plant senescence was mistaken for stem rot damage later in the season. Numbers of sclerotia formed per tiller increased up to 77 days after flowering (a month after normal harvest), the last date sampled. The increases for all cultivars except M-101 and L-202 were small between 35 and 77 days after flowering. M-101 and L-202 were the most susceptible to stem rot in this trial and in the time-of-rating trial.

Greenhouse fertilizer trials. Higher initial (21 days after seeding) nitrogen rates significantly ($P = 0.02$) increased stem rot rating in 1984 but less so in 1985 ($P = 0.26$, Table 1), when disease severity was lower. Cultivars differed significantly in stem rot rating in both years. M-101 was more susceptible (8.5 and 7.2 average in 1984 and 1985, respectively) and M-201 was less susceptible (6.6 and 5.9 in 1984 and 1985, respectively) to stem rot. There were no nitrogen × cultivar interactions.

Correlation of greenhouse and field ratings over years. Correlations of greenhouse with field ratings ranged from $r^2 = 0.88$ ($P = 0.06$) to 0.94 ($P = 0.03$) for within-year comparisons, and from 0.36 ($P = 0.4$) to 0.94 ($P = 0.03$) for between-year comparisons (Table 2). Correlations of between-year field comparisons ranged from $r^2 = 0.59$ ($P = 0.29$) to 0.99 ($P = 0.01$). Correlations of between-year greenhouse comparisons ranged from $r^2 = 0.47$ ($P = 0.31$) to 1.0.

DISCUSSION

Rating cultivars 42 days after flowering resulted in the largest differences among cultivars and the greatest disease severity (and hence sclerotium formation). Plants approach or have reached harvest maturity by this time and are apparently less able to defend themselves

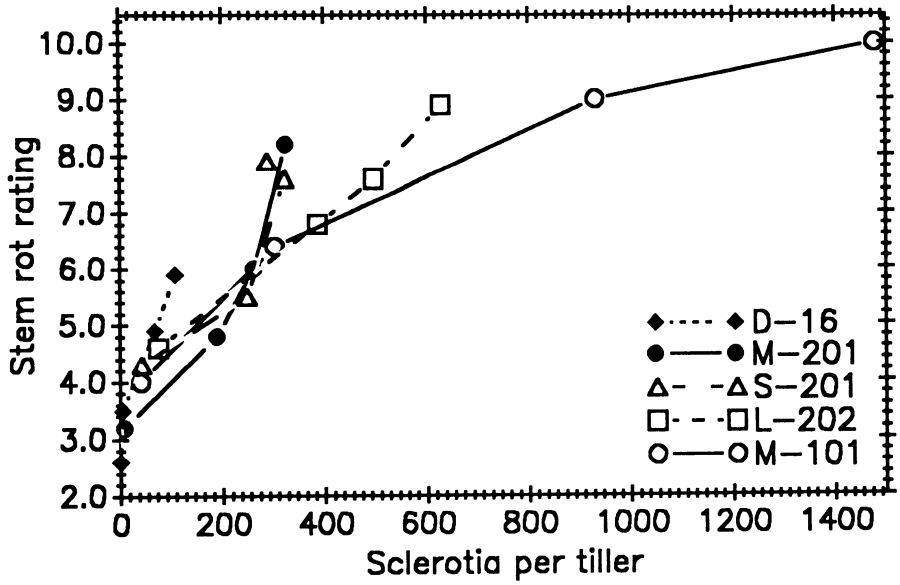

Fig. 2. Correlation of stem rot rating with number of sclerotia per tiller in 1987 for five rice cultivars (D-16, M-201, S-201, L-202, and M-101). Each cultivar was sampled at 0, 21, 35, and 77 days after flowering. For all cultivars, $r^2 = 0.60$, $P < 0.001$. The regression equation is: stem rot rating = 0.0079 (sclerotia per tiller) + 3.79. The r^2 for individual cultivars ranged from 0.52 to 0.77, $P < 0.001$. Ratings based on a 1–10 scale.

Table 1. Effect of nitrogen rate on stem rot severity in the summer greenhouse, 1984 and 1985

Rate[d] (g of N/pot)	Mean stem rot rating[a]					
	1984[b]			1985[c]		
	M-101	M-201	Average	M-101	M-201	Average
0.21	8.0	6.2	7.1	6.9	5.8	6.4
0.53	8.6	6.5	7.6	7.2	6.0	6.6
0.85	8.9	7.2	8.0	7.5	6.0	6.8

[a]Rated on a 1–10 scale.
[b]Significant differences occurred both in stem rot rating among nitrogen rates (LSD = 0.65, $P = 0.05$), and between cultivars ($P < 0.001$). There was no cultivar × nitrogen rate interaction.
[c]Differences between cultivars were significant ($P < 0.001$) but among nitrogen rates only at a lower level (LSD = 0.38, $P = 0.26$). There was no cultivar × nitrogen rate interaction.
[d]Nitrogen applied 20–21 days after seeding. Lowest nitrogen rate applied was 0.21 g of N per pot as 16-20-0 (15.8 kg of N/ha), with additional amounts as ammonium sulfate (0.53 g of total N per pot = 40.0 kg of N/ha or 0.85 g of total N per pot = 52.2 kg of N/ha). In addition, top-dressing occurred 38 and 35 days after seeding with ammonium sulfate (0.32 g of N per pot or 24.2 kg of N/ha) and plants were inoculated 48 and 45 days after seeding in 1984 and 1985, respectively.

Table 2. Correlations of summer field (F) and greenhouse (GH) stem rot ratings, 1984 to 1988[a]

			1984		1985		1986		1987		1988	
			F	GH	F	GH	F	GH	F	GH	F	GH
1984	F	r^2	···	0.89	0.84	0.36	0.67	0.87	0.67	0.87	0.59	0.92
		P	···	0.06	0.08	0.40	0.18	0.07	0.18	0.06	0.29	0.04
	GH	r^2		···	0.84	0.52	0.90	0.91	0.81	0.92	0.79	1.00
		P		···	0.08	0.28	0.05	0.04	0.10	0.04	0.11	0.00
1985	F	r^2			···	0.88	0.97	0.92	0.99	0.93	0.94	0.80
		P			···	0.06	0.02	0.04	0.01	0.04	0.03	0.10
	GH	r^2				···	0.76	0.72	0.90	0.71	0.80	0.47
		P				···	0.12	0.15	0.05	0.16	0.11	0.31
1986	F	r^2					···	0.88	0.92	0.89	0.97	0.86
		P					···	0.06	0.04	0.06	0.01	0.07
	GH	r^2						···	0.94	1.00	0.77	0.90
		P						···	0.03	0.00	0.12	0.05
1987	F	r^2							···	0.94	0.87	0.77
		P							···	0.03	0.07	0.12
	GH	r^2								···	0.78	0.91
		P								···	0.12	0.14
1988	F	r^2									···	0.91
		P									···	0.04

[a]Data collected for cultivars M-101, M-201, L-202, and D-16.

against stem rot. Results from California (11) correlating disease ratings with yield reduction suggest ratings taken at this time accurately reflect economic damage. For these reasons, ratings for disease resistance should be taken at maturity.

Correlations of stem rot rating with number of sclerotia formed per tiller were higher for individual cultivars than for the cultivars taken together. This indicates cultivar specificity for sclerotium development. In 1986, disease severity and sclerotium development were not related in the resistant line D-16. Because disease severity was lower in 1986, D-16 tissue conducive to sclerotium formation may not have been infected in most tillers. Rate and time of disease development also differed among cultivars. Both stem rot rating and number of sclerotia formed were greatly reduced in the resistant line D-16. Sclerotia are the propagules primarily responsible for initiating disease within a growing season and persist in the soil for many years (3,4). Because stem rot rating was positively correlated in this study with the amount of sclerotia formed, it may also be useful in predicting the potential damage a cultivar will sustain with monoculture over time.

Greenhouse screening techniques are important because inoculation, temperature, plant identity, and weeds can be more carefully controlled, and one more generation per year can be raised in the greenhouse than in the field. Nitrogen fertilization rate was an important factor in disease development. Higher initial nitrogen rates increased disease severity but did not increase differences in stem rot rating between susceptible and resistant cultivars (M-101 and M-201, respectively). In view of this and because increased nitrogen rates promote algal and insect proliferation and decrease pollen viability of plants used for crossing (J. Oster, *unpublished*), the initial rate of 0.21 g of nitrogen should be used for disease resistance screening in the greenhouse.

Despite differences in greenhouse and field culture, high correlations were noted among results obtained in each environment and within each environment over time. Greenhouse screening techniques appear to be adequate for use in a breeding program; however, only a few cultivars were tested in this study. These cultivars represented a range in stem rot susceptibilities but not necessarily the full range of genetic variability for other traits influencing stem rot susceptibility present in the breeding program.

Successful breeding for disease resistance requires production of repeatable severity levels that allow expression of a full spectrum of resistance levels under realistic production conditions. In field studies, nitrogen rates that produce optimum yields for most current cultivars in California were chosen. Most nitrogen was applied preplant and incorporated to increase retention and promote disease severity. More nitrogen was applied to flushed fields than to permanently flooded fields to compensate for nitrogen loss through volatilization (8,12). Plants were inoculated at panicle initiation (adjusted to early-maturing cultivars when very early and late-maturing cultivars were also in a test), because this produced maximum disease (10,11). Sheaths (the primary infection court) had emerged from the water by this time and allowed contact by floating sclerotia. This was important because the water level could not be lowered to expose leaf sheaths and still maintain sufficient depth to suppress weeds. Inoculation rates were chosen to produce infection of every tiller. Inoculum production techniques resulted in efficient production of large amounts of inoculum necessary in a high-volume breeding program. Inoculation techniques described here allowed rapid and uniform application of sclerotia to plants.

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