Decline in Numbers and Inoculum Potential of *Sclerotium oryzae* in Field Soil

W. W. Bockus and R. K. Webster

Assistant professor, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506; and professor, Department of Plant Pathology, University of California, Davis, CA 95616.

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ABSTRACT

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A calculated half-life of 1.9 yr was obtained for sclerotia of *Sclerotium oryzae* rototilled into field soil to an 18-cm depth with or without an amendment of dried, noninfested rice residue. During the first 4 wk, living sclerotia incorporated in soil germinated 80% when recovered from soil and placed on water agar. Germination of sclerotia on water agar declined to 20% after 6 mo and thereafter stabilized between 20 and 30%. Treatment with 0.5% NaOCl indicated that loss of germinability was due to dormancy but not to complete death of sclerotia. Number of sclerotia per gram of soil that germinated when placed on water agar amended with streptomycin sulfate and penicillin G was a good measure of the inoculum potential of *S. oryzae*. Initially the inoculum potential of freshly-produced sclerotia incorporated in field soil declined rapidly due to the rapid loss in the ability of sclerotia to germinate, but the total numbers of sclerotia decreased only slightly. After the rapid decline, inoculum potential stabilized and thereafter is expected to be influenced largely by a decline in actual numbers of sclerotia. Because the decline in numbers of sclerotia of *S. oryzae* was relatively slow (half-life = 1.9 yr), alternate year cropping of rice would not be expected to control stem rot.

Additional key words: Magnaporthe salvinii.

Magnaporthe salvinii Krause and Webster (*Sclerotium oryzae* Catt.) causes stem rot of rice (*Oryza sativa* L.). Soilborne sclerotia serve as the overwintering propagules for survival of the fungus. After fields are flooded, sclerotia float to the surface of paddy water. Floating sclerotia contact emerging rice plants and germinate, and the mycelium infects tillers at the water line (10). Sclerotia are produced on or in infested tissues as the rice plants mature and either remain in crop debris or are dislodged and scattered on the soil surface during harvest. Subsequent tillage incorporates the sclerotia into the soil. Efforts to control stem rot have been directed toward minimizing inoculum by cultural practices (14–16).

Park and Bertus (11) recovered viable sclerotia after 190 days from air-dry soil and after 133 days from moist rice-field soil. Nisikado and Hirata (9) reported viability of air-dry sclerotia after 3 yr in a 20 C incubator. Tullis and Cralley (12) buried infested rice straw and sclerotia 10–15 cm deep in field soil and reported that 3.7% of the sclerotia were viable after 6 yr. These studies did not, however, distinguish between decline in numbers of sclerotia and decline in percentage germination (germinability) after various periods of time. Such information is essential because there are correlations (8,13,16) between viable sclerotia per gram soil, disease severity, and yield loss due to stem rot. Although Webster et al (14) reported the effect of various residue management practices on vertical distribution and survival of sclerotia of *S. oryzae* under continuous rice culture, decline in sclerotial numbers and changes in inoculum potential of *S. oryzae* in field soil in the absence of the living host have not been reported.

MATERIALS AND METHODS

Measurement of inoculum decline in the field. Sclerotia of six isolates of *M. salvinii* produced on a sterile rice-rice hull mixture according to Krause and Webster's method (7) were used to infest field soil artificially. The six isolates (B-6, D-6, D-15, D-19, D-43, and D-55) represented both mating types and varied in sclerotia production, growth rate, and virulence (3). Equal quantities of sclerotia of each isolate were mixed and incorporated in 3 × 3 m areas in a field. Each area (basin) was surrounded by a dirt levee 30 cm high × 1 m wide. The experimental field had never been cropped to rice and no sclerotia of *S. oryzae* were detected in the soil. The sclerotia were sprinkled on the soil surface and thoroughly mixed into the soil (Yolo sandy loam) to an 18-cm depth using a hand-operated rototiller. Propagules were introduced at a rate of 3–5 sclerotia per gram of soil, which corresponds to a moderate infestation level (14,16). There were three treatments of sclerotia and four replicate basins for each treatment. Treatments included: (i) sclerotia incorporated in the soil along with 4.5 kg of chopped, dried, noninfected rice residue; (ii) sclerotia rototilled into the soil without residue; and (iii) sclerotia killed with propylene oxide in a sealed (24 hr) quart mason jar before incorporation. Four noninfested basins served as controls. The 16 basins in the field were in a randomized complete block design. The field was occasionally sprinkler-irrigated to supplement the rainfall and provide a total average annual precipitation of 45–50 cm.

During 1.5 yr the basins were sampled periodically and the number of sclerotia per gram of soil determined. At each sample date 30 soil cores (1.8 cm in diameter and 15 cm deep) were collected at random from each basin with a standard soil-core sampler. The 30 cores were bulked in a paper bag, air-dried, and mixed, and then four 50-g subsamples were taken from each bag and the number of sclerotia determined. Krause and Webster's extraction method (7) enables the recovery of 90–95% of the sclerotia in a soil sample.

Determination of percent germination of sclerotia. Viability of sclerotia has been determined by placing them on water agar (7,14), where germinated sclerotia produced the conidial state of *M. salvinii* (*Nakatea sigmoidea* (Cav.) Hara). For this study percent germination on water agar (1.5% Difco Agar Flake) amended with streptomycin sulfate and penicillin G each at 100 ppm (WA+) was used. Sclerotia were placed on WA+ in petri dishes and incubated 30 cm below continuous fluorescent lights (about 690 lx) for 14 days at 24 ± 2 C. Sclerotia tested for germination were extracted from soil from basins with sclerotia alone (treatment ii). On each sample date 200 sclerotia from each replicate basin were placed on WA+. To study the effect of surface sterilization on percent germination, some sclerotia were shaken for 30 min in a 0.5% aqueous NaOCl solution, rinsed in sterile distilled water, and placed on WA+.

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In another study sclerotia were extracted from soil from a grower's rice seedbed in 1977 before the field was flooded. The sclerotia were plated directly onto WA+ or were exposed to the NaOCl treatment before being placed on WA+.

Tests for infectivity of sclerotia. To determine the correlation of declines in percent germination of sclerotia on WA+ and in infectivity, samples of sclerotia with different percentages of germination on WA+ were used to inoculate rice plants grown in the greenhouse. Soil from basins with sclerotia alone (treatment ii) was sampled 1 wk, 1.5 mo, 4.5 mo, and 7 mo after incorporation of sclerotia in the soil.

Rice plants of the M-5 cultivar (California Cooperative Rice Research Station, Biggs, CA 95917) were grown in the greenhouse in 20-L plastic buckets and thinned to five plants per bucket 14 days after seeding (8). A glass needle was used to place a sclerotium on each tiller at the water line 65 days after planting. The sclerotia were held against the tillers by the water meniscus. A drip-irrigation system was used to maintain a constant water level in each pot so that the sclerotia remained at the tiller-water interface during the 3-wk experiment. For each sample date, 200 tillers were inoculated and 50 noninoculated tillers served as controls. For each sampling 200 sclerotia also were placed on WA+. The percentage of infected tillers was determined after 3 wk. Infections appeared as small, black, elongated lesions at the water line (8).

In another experiment, sclerotia were placed on top of 7-cm, freshly cut, 65 day old tillers in petri dishes. Five sclerotia were placed on top of each of five green rice pieces in a petri dish lined with moist filter paper. Sclerotia were placed far enough apart on the tiller pieces so that stem rot lesions from adjacent sclerotia did not coalesce.

For each of the four sample dates (1 wk, 1.5 mo, 4.5 mo, 7 mo), 100 sclerotia were obtained from treatment (ii) and placed on the tiller pieces. For each sample date 200 sclerotia also were placed on WA+. All petri dishes were incubated at 24 ± 2 C under continuous fluorescent lights for 12 days, at which time the number of stem rot lesions initiated by single sclerotia were counted.

RESULTS

Inoculum decline in the field. Numbers of sclerotia declined in all three treatments. The rate of decrease was similar whether sclerotia were living or dead when incorporated in soil (Fig. 1). The numbers of sclerotia decreased approximately 35% during the 1.5-yr experiment.

Percent germination of sclerotia. Sclerotia introduced without residue into the soil in the fall of 1976 initially germinated about 80% on WA+ (Fig. 2). During the overwintering period (November 1976–May 1977) percent germination was progressively less with each sampling. Thereafter percent germination stabilized between 20 and 30% (May 1977–January 1978). Although the data are not presented, sclerotia introduced in the soil together with noninfested rice residue followed an essentially identical pattern of progressively less and then stabilization of percent germination. This decline in germination on WA+ was not due to complete death of sclerotia because a 30-min exposure to 0.5% aqueous NaOCl before plating on WA+ resulted in 75% or more germination of the sclerotia regardless of the sampling date (Fig. 2). In another test 400 sclerotia extracted from soil from a grower's field germinated 25.5% on WA+, but 400 sclerotia extracted from the same soil and exposed to NaOCl before being plated on WA+ germinated 72.5%.

Percent infectivity vs. inoculum potential Two hundred sclerotia from each of the four germination classes produced 17.0, 16.0, 10.0, and 4.5% infection, respectively, when inoculated individually onto rice tillers (Fig. 3A). All noninoculated tillers remained healthy. The correlation coefficient for percent germination on WA+ vs. percent infection was 0.96.

At each of the four sample dates 100 sclerotia produced 34, 19, 18, and 7 infections, respectively, when placed on pieces of green rice tillers in petri dishes and incubated for 12 days (Fig. 3B). Correlation coefficient for percent germination on WA+ vs. percent infection was 0.94. Both tests were repeated and again produced a high coefficient of correlation.

DISCUSSION

For the conditions of this study the calculated apparent half-life (17) for decomposition of *S. oryzae* sclerotia in soil is 1.9 yr. Numbers of sclerotia decrease presumably because of degradation by soil organisms. Sclerotia killed before incorporation in soil decreased at the same rate as living sclerotia indicating that persistence of sclerotia is passive and probably due to the resistance of the sclerotial rind to microbial degradation. However, Ferguson (2) reported that killed sclerotia of *Sclerotinia sclerotiorum* and *Sclerotium delphinii* were colonized by various soil organisms but

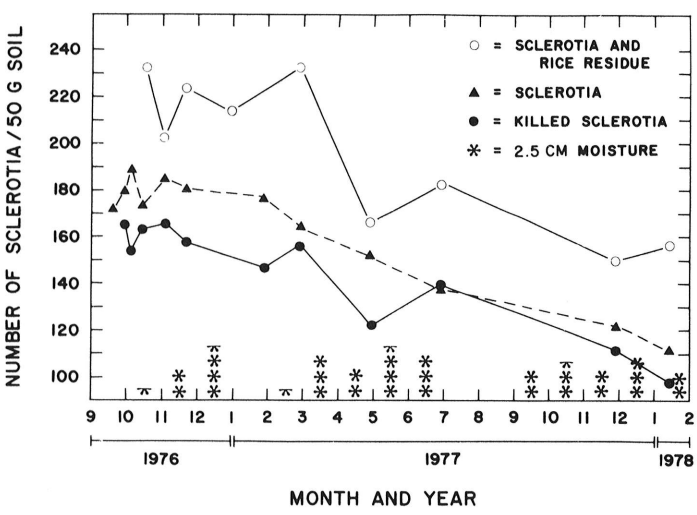

Fig. 1. Decline in numbers of sclerotia of *Sclerotium oryzae* after initial infestation of field soil. Initially sclerotia either were incorporated in the soil with healthy rice residue (open circles), alone (triangles), or dead, ie, exposed to propylene oxide and then incorporated (closed circles). Each point represents the mean number of sclerotia extracted from 16 50-g soil samples from four replicate areas. LSD ($P = 0.05$) = 46.6 for sclerotia incorporated with rice residue, 17.3 for sclerotia incorporated alone, and 27.8 for dead sclerotia.

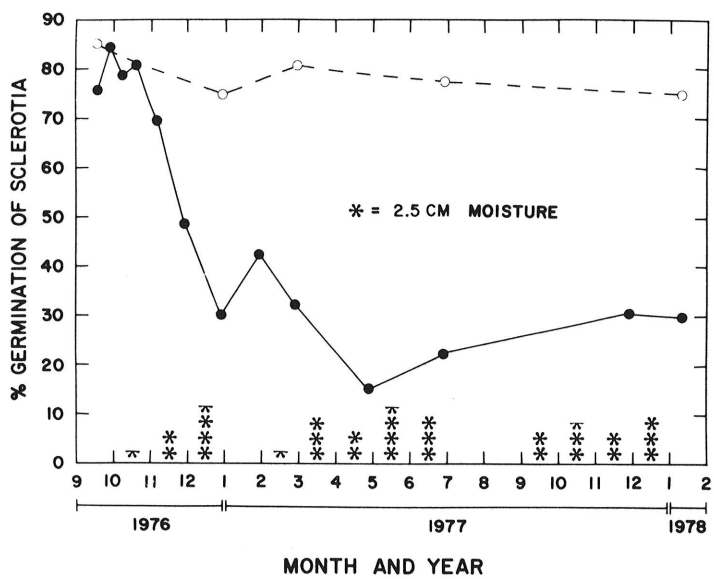

Fig. 2. Decline in percent germination of sclerotia of *Sclerotium oryzae* after various times in field soil. Sclerotia were incorporated in field soil, periodically extracted, and either washed in a NaOCl solution before being placed on water agar (open circles) or plated directly onto water agar (closed circles). Each point represents the percent germination of 800 sclerotia extracted from four replicate areas. LSD ($P = 0.05$) = 7.5.

that living sclerotia were rarely colonized. It is not known if killed and living sclerotia of *S. oryzae* are differentially colonized by soil organisms, what effect colonization has on the recoverability of sclerotia from soil, or which organisms are involved in sclerotial degradation.

Bockus et al (1) reported that the competitive saprophytic ability of *S. oryzae* derived from sclerotia was restricted in field soil. In those tests *S. oryzae* did not colonize rice residue or organic matter in soil and produce additional sclerotia. Our results confirm this earlier finding; living sclerotia incorporated in soil along with chopped, noninfested rice residue decreased in numbers at the same rate as living or dead sclerotia incorporated alone.

Webster et al (14) reported that sclerotia recovered from standing rice stubble and straw in the fall immediately after harvest germinated 76–92% on water agar. Others reported that sclerotia recovered from soil from a rice field in the spring, after they had overwintered, germinated only 25% on water agar (5,7). Keim and Webster (6) reported a significant reduction in percent germination of sclerotia of *S. oryzae* after 29-day incubation in moist soil in petri dishes. Results from this study also showed that a high percentage of sclerotia incorporated in an experimental field soil for several months did not germinate on WA+. This decrease in percent germination was due to dormancy of a high percentage of the sclerotia because treatment with NaOCl increased germination from about 20% to at least 75%. It is not known if sclerotia in the field are later released from this dormancy.

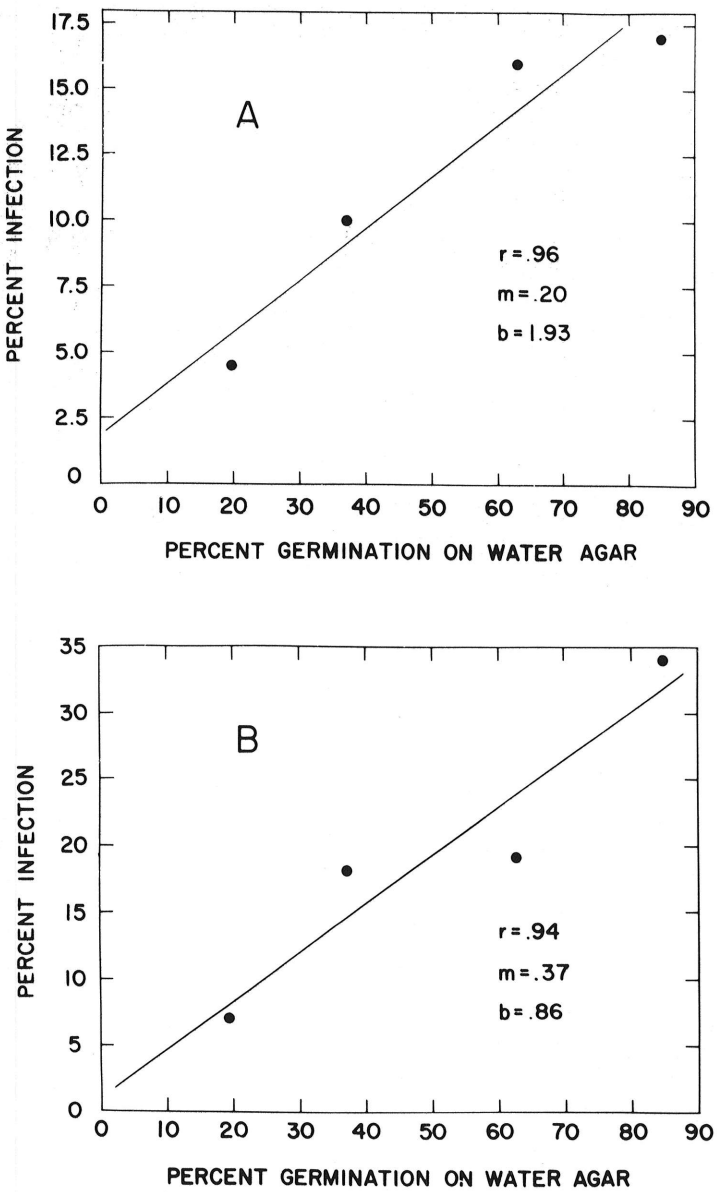

Fig. 3. Correlation of percent sclerotia of *Sclerotium oryzae* initiating stem rot infections with percent germination of sclerotia on water agar. Samples of sclerotia that germinated different percentages on water agar were either individually inoculated onto rice tillers in the greenhouse (A) or placed on cut green rice tillers in petri dishes in the laboratory (B).

Percent germination of sclerotia placed directly on WA+ was highly correlated with percentage of sclerotia that initiated stem rot disease. However, only one-fifth as many sclerotia initiated disease, compared with percent germination on WA+. Environmental conditions as well as ability to germinate apparently influence infection. However, percent germination of sclerotia placed directly on WA+ can be used to indicate the "relative infectivity" of different samples of sclerotia.

The term "inoculum potential" has been defined by Garrett (4) as "energy of growth of a pathogen available for infection of a host at the surface of the host organ to be infected." He further stated that the nutritional status of potentially infective units and their numbers influence the inoculum potential (4). When comparing soil samples, therefore, germinated sclerotia per gram of soil is a useful estimate of the inoculum potential of *S. oryzae* because it takes into account the number of sclerotia in the soil and the relative ability of *S. oryzae* to initiate disease from those sclerotia. The number of germinated sclerotia per gram of soil in the seedbed has been correlated with disease severity and yield loss (8,13).

Based on percent germination, there was an initial rapid decline in the inoculum potential of freshly-produced sclerotia incorporated in field soil (Fig. 2). This rapid decline occurred even though actual numbers of propagules in the soil were virtually unchanged. After the initial rapid decline, inoculum potential stabilized at a level where a relatively low percentage of sclerotia were functional at any time. During this period the change in inoculum potential was slow and, assuming no subsequent large change in percent germination of sclerotia occurs, further decreases in inoculum potential should be due to a decrease in actual numbers of sclerotia per gram of soil.

The initial rapid decline in inoculum potential that occurred during the overwintering between rice crops would influence the amount of disease in continuous rice culture. The slower subsequent decrease in actual numbers of sclerotia should, however, be more important to the effectiveness of crop rotation for controlling stem rot of rice. Reports that alternate year rice cropping is not an effective control measure for stem rot (12,14) are supported by our results showing that sclerotia of *S. oryzae* persist (half-life = 1.9 yr) in field soil. Thus short-term rotations of rice with nonhost crops would not be expected to lower the inoculum potential of *S. oryzae* below the economic threshold (13).

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