Effect of Soil Moisture and Temperature on Viability of Sclerotia of Sclerotium oryzae

R. Keim and R. K. Webster

Department of Plant Pathology, University of California, Davis 95616. Present address of senior author: Superintendent, University of California, South Coast Field Station, 7601 Irvine Blvd., Santa Ana 92705.

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ABSTRACT

Alternate wetting and drying of sclerotia of *Sclerotium oryzae* resulted in substantial loss of sclerotial weight accompanied by reductions in viability. Viabilities of sclerotia recovered from wet soils after incubation at 24 C were significantly lower than the viabilities of those incubated at 1 C and those incubated at 24 C after they were recovered from soils that had been allowed to dry. They were also lower than viabilities of sclerotia subjected to alternate wetting and drying. It would appear that a soil fungistatic factor was imbibed by sclerotia and retained after their recovery from soil.

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Sclerotia of *Sclerotium oryzae* Catt. are formed in tissues of rice (*Oryza sativa* L.) infected with stem rot disease near the time of maturity, and remain in rice debris and on the soil after harvest. Subsequently, sclerotia become incorporated into the soil at various depths and at various times and serve as the primary means of overwintering and survival. Evidence indicated that a direct relationship existed between numbers of

viable sclerotia found in rice field soils in the spring before planting, and the severity of stem rot disease of rice at the end of the same growing season (8). Sclerotia recovered from rice field soil in the fall after the soil had been under water 4 months during production of a rice crop, were found to have an average viability of 24% (5). Viability of sclerotia recovered in the spring before seeding was 32% (3). The viability of sclerotia which float to the surface of paddy water and act as the primary inoculum for stem rot disease of rice is therefore an important key to possible methods of biological control of the disease. This paper reports on some effects of soil moisture on sclerotial viability with relationships to loss of sclerotial endogenous reserves and possible biotic inhibitory factors.

MATERIALS AND METHODS.—Two lots of sclerotia produced on inoculated rice hull cultures (5), and subsampled at various intervals from 1 to 8 months while stored at 1 C, were found to be 88% and 100% viable, respectively. The soil was obtained from a field on which rice had been grown for 5 years free from stem rot disease. Rice straw from the crop grown in the field was chopped and incorporated into the soil (3.5 mg per g of dry soil) to simulate field incorporation. Sclerotia were introduced into the soil in petri dishes by placing 200 ± 25 in each empty, sterile dish and then covering with soil.

Moisture content of soil is expressed as P_w (1), which is percent moisture on an oven-dry basis (constant weight at 105 C). The field capacity of the soil used in these tests was determined by the pressure plate technique at one-third bar pressure to be 27.5 P_w. Moisture contents of soils incubated in petri dishes were established and maintained within desired limits as follows: (i) the soil was air-dried in the laboratory to a constant P_w of 5; (ii) 60 g of air-dry soil (5 P_w) were placed in each petri dish; (iii) sufficient distilled water was added to each dish to wet the soil to 40 P_w in all cases except 60 P_w tests; (iv) then dishes were allowed to dry until the gross weight of each equalled that calculated for the desired P_w; (v) constant P_w values of soils in the dishes were maintained by weighing the petri dishes at regular intervals and adding distilled water uniformly over the surface of the soil from a pipette to compensate for that lost by evaporation.

Introduced sclerotia were recovered from soil for viability tests (5) by blending 60 to 100 g of soil with ca. 240 ml water for 20 sec, screening out large debris on a 7.9 mesh per cm screen and collecting the remaining particles on a 39.4 mesh per cm screen. The latter were then washed into a beaker and the particles that floated (including buoyant sclerotia) were vacuumed into an air suction flask with a glass dropper connected to rubber tubing and collected on filter paper in a Büchner funnel. Sclerotia which were not buoyant during the initial extraction were recovered by filtering out all the remaining particles from the 39.4 mesh per cm screen which did not float, allowing them to dry on filter paper and stirring them back into a beaker of water. The sclerotia which floated to the surface were collected as previously described. All sclerotia were allowed to air-dry before being tested for viability.

Sclerotial viability was determined by the percent germination of sclerotia incubated for 14 days on water agar (1.5% Difco Agar Flake) at 24 ± 2 C (5).

TABLE 1. Buoyancy and viability of sclerotia of *Sclerotium oryzae* incubated at 24 ± 2 C and recovered from moist soils which were not allowed to dry before sclerotial recovery

Adjusted soil moisture (P_w)	Recovered sclerotia	
	Number buoyant[a]	Percent viability[a]
5-27.5[b]	158	62.2
27.5	20	16.8
40.0	7	12.3
60.0	4	0.5

[a]Values are averages of 24 replicates with 160 ± 25 sclerotia recovered from 200 ± 25 introduced into each petri dish; LSD ($P = 0.05$) = 12.3 for buoyant sclerotia and 8.8 for percent viability.

[b]Soils with moisture of 5-27.5 P_w were incubated in open petri dishes and allowed to dry to 5 P_w and rewetted to 27.5 P_w daily to simulate field conditions.

RESULTS.—Sclerotia were incubated at 24 ± 2 C for various lengths of time from 2 to 10 weeks at different levels of soil moisture content. The sclerotia were recovered, counted and tested for viability (Table 1). Only those sclerotia which were recovered by the first flotation were considered buoyant. The buoyancy of the incubated sclerotia recovered from those soils which were not permitted to dry between incubation and sclerotia recovery was inversely related to soil moisture content, as was sclerotial viability.

Because of the correlation between loss of sclerotial viability and increasing levels of soil moisture, sclerotia were washed in water to determine what effects this might have on their weight and viability. Dry sclerotia were added to sterile distilled water (500 mg/125 ml) in 250 ml flasks, washed for 24 h on a rotary shaker, collected on filter paper in a Büchner funnel and allowed to air-dry for 24 h after which a 0.5 mg subsample of each lot was tested for viability. Results of 10 successive cycles of the washing process indicated that the sclerotia lost a total of 50% of their original weight during the entire process and generally decreased in viability with each successive washing and loss of weight (Table 2). Their final viability after the 10th washing during which they essentially lost no further weight was 23%.

TABLE 2. Viability and weight of sclerotia of *Sclerotium oryzae* after alternate cycles of washing and air-drying

Cycles of washing and drying	Sclerotia	
	Viability[a] (%)	Weight[a] (mg)
0	100	500 (initial weight)
1	99	405
2	79	376
3	71	340
4	51	319
5	48	306
6	36	288
7	38	276
8	26	269
9	30	252
10	23	251

[a]Values are averages of four replications; r = 0.834.

The results reported in Table 2 supplied evidence to explain why sclerotial viability was affected least by the drier soil (5 - 27.5 P_w) in Table 1, but they did not account for the low viability of sclerotia laboratory-incubated in soils with moisture contents of field capacity or greater. In another series of tests, sclerotia were incubated for 29 days in moist soils which were then allowed to dry in open petri dishes until soil weights were stable (ca. 5 P_w) before recovery of sclerotia. The results indicated that the viability of sclerotia under those conditions was not significantly different at the different incubation moisture levels with the exception of that found at 60 P_w (Table 3). Sclerotial viabilities resulting from the two different methods of handling soil moisture prior to sclerotial recovery are compared in Fig. 1.

Sclerotia were then incubated for 2 or 14 days in soil with 40 P_w at 1 C or 24 C and recovered without allowing the soil to dry. The results showed that in as little as 2 days the viability of sclerotia incubated at 24 C was significantly lower than at 1 C (Table 4).

DISCUSSION. — The loss of buoyancy of sclerotia during incubation in wet soils suggested that they imbibed water after resistance to surface wetting was overcome, and because this occurred while incubating at 1 C the cause was not necessarily biotic. This was further substantiated by stirring dry sclerotia into water to which a surfactant (Tween 20) had been added. After 5 min of stirring they were still afloat, but 48 h later they had sunk, indicating that more than just surface wetting was required. Interstices or pores in the surface structure of sclerotia have been demonstrated (4).

The loss of sclerotial weight and viability resulting from repeated washings appeared to account for the low viability which was characteristic of sclerotia recovered from wet field soils, but not for the extremely low viability of sclerotia recovered from wet soils in the laboratory (Table 1), particularly when P_w was 60. The comparison of viabilities of sclerotia recovered from wet soils and those recovered from wet soils which had been allowed to dry (Fig. 1) suggests that something from the soil present in the imbibed water inhibited sclerotial germination and that when allowed to dry in the soil the sclerotia lost both the imbibed water and the inhibitory factor. However, when sclerotia were recovered from wet soils at 1 C, their viability averaged as high as the viability of those recovered from wet soils at 24 C which had been allowed to dry before recovery, suggesting that some biological activity was responsible for the presence of the inhibitory factor. It should be emphasized that the recovery of sclerotia from soil involved 20 sec of blending of the soil sample in water, repeated washing during the screening process and vigorous stirring in ca. 300 ml of water to allow the buoyant sclerotia to float, indicating that the inhibitory factor was tightly held within the surface structure of the sclerotia.

The phenonmenon of soil fungistasis (2,6,7) has been described as a property of soils which prevents or inhibits the germination of fungal propagules and has been shown to be biotic in origin. We know of no other report in the literature in which the factor considered responsible for soil fungistasis had been significantly correlated with the ability of fungal propagules to germinate after removal from soil.

There is an aspect of this study which may be important

TABLE 3. Buoyancy and viability of sclerotia of *Sclerotium oryzae* incubated at 24 ± 2 C in moist soils and recovered after the soils were allowed to dry to 5 P_w

Soil moisture		Recovered sclerotia	
During recovery (P_w)	At time of incubation (P_w)	Buoyant (no.)	viability[a] (%)
11.0	5	154	69
16.0	5	158	59
27.5	5	190	56
40.0	5	219	45
60.0	5	212	18

[a]Values are averages of 4 replicates of sclerotia recovered from 200 ± 25 introduced into each petri dish; LSD ($P = 0.05$) = 16.1.

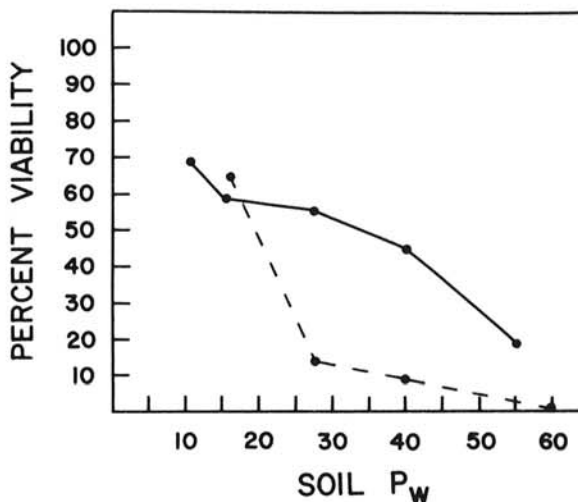

Fig. 1. Percent viability of sclerotia of *Sclerotium oryzae* incubated in wet soil at 24 ± 2 C and recovered after the soil was allowed to dry (solid line) compared with sclerotia recovered before the soil was allowed to dry (broken line).

TABLE 4. Viabilities of sclerotia of *Sclerotium oryzae* incubated for 2 or 14 days at 1 C or 24 C and recovered from soils with 40 P_w which were not allowed to dry before sclerotial recovery

Incubation treatment		Sclerotia recovered		Total
		First	Second	sclerotial
Temp (C)	Time (days)	flotation (no.)	flotation[a] (no.)	viability[b] (%)
1	2	9	124	41.8
1	14	14	138	52.4
24	2	3	181	14.4
24	14	3	142	13.4

[a]After air-drying particles, etc. that did not float in the first recovery.

[b]Values are averages of 5 replicates of sclerotia recovered from 200 ± 25 introduced into each petri dish; LSD ($P = 0.05$) = 24.4 and LSD ($P = 0.01$) = 33.7.

regarding possible biological control of stem rot disease of rice. The samples of field soil from which sclerotia were recovered and reported to have viabilities averaging 24% (5) and 32% (3) were from rice fields in which stem rot disease was endemic, while the soil used in the laboratory tests was taken from a rice field where rice free of the disease was grown for 5 years. This was necessary to assure experimental soil free of sclerotia. It is possible that the nature of the fungistatic factor in the latter soil may be responsible for the absence of disease in that field. Studies are being conducted to critically compare these soils and also to determine which organisms present in the latter may be responsible for the persistent fungistasis of the sclerotia of *Sclerotium oryzae* reported here.

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