The Competitive Saprophytic Ability of Sclerotium oryzae Derived from Sclerotia

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ABSTRACT

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Radioactive (^{14}C-labeled) sclerotia of *Sclerotium oryzae* were incubated in moist soil in petri dishes to determine their ability to produce new sclerotia saprophytically. Soil used for the study was from two locations on which rice had been grown for a number of years. Since the soils were infested with *S. oryzae* the naturally occurring sclerotia were removed by sieving before the introduction of labeled sclerotia. Treatments included both autoclaved and nonautoclaved soil, with or without an amendment of dried, noninfected, nonsterilized rice sheaths. Sclerotia produced in the soil by *S. oryzae* as a result of saprophytic colonization of organic material were not labeled and thus were distinguishable from the labeled sclerotia originally introduced. The saprophytic ability of *S. oyrzae* was too low to enable it to use residual organic material in either nonautoclaved or autoclaved soil. In autoclaved soil amended with rice sheaths the sclerotia germinated, the fungus colonized the sheaths, and the number of sclerotia increased. In nonautoclaved soil amended with rice sheaths a few new sclerotia were produced as a result of colonization of the sheaths but they did not contribute significantly to the population of sclerotia in the soil. In another experiment, nylon mesh bags filled with soil containing chopped rice sheaths and radioactive sclerotia were buried in moist field soil for 5 wk. No new sclerotia were recovered which were produced as a result of saprophytic colonization of the sheaths. The use of radiolabeled sclerotia, as described here, is considered a useful aid in following populations of sclerotia in the soil.

Additional key words: rice stem rot, *Magnaporthe salvinii*.

Rice (*Oryza sativa* L.) stem rot, caused by *Sclerotium oryzae* Catt. [ascosporic state: *Magnaporthe salvinii* (Cattaneo) Krause and Webster], is endemic throughout northern California rice-growing areas (10). Sclerotia of the fungus serve as the primary inoculum. They float to the surface of rice paddy water, germinate, and the mycelium infects rice plants. Sclerotia also serve as the overwintering stage for survival of *S. oryzae*. A direct relationship exists among the number of viable sclerotia per gram of soil in the spring before planting, disease severity, and subsequent yield loss due to stem rot (11). Thus, the production of sclerotia by *S. oryzae* plays a key role in rice stem rot. Sclerotia are produced on or in infected rice tissues as the rice plants mature (9). They then are incorporated into the soil along with plant residues by various tillage methods. In the laboratory, sclerotia also are formed on or in infected rice residue that has been incorporated into moist soil (5).

Griffin (3) defined competitive saprophytic colonization as the saprophytic colonization of a substrate by a mixed population. Thus, saprophytic colonization of a substrate in the soil involves the interactions among different organisms that are colonizing the substrate. Griffin (3) refers to antagonistic interactions as exploitation, competition, and antibiosis.

In California, approximately 9,000 to 16,000 kg of rice residue per hectare are produced each year. It follows that, in an infested field, tillage practices would liberate sclerotia from infected tissues and distribute the sclerotia throughout the soil along with much noninfected rice residue. Thus, there is the potential for saprophytic colonization of rice residue by *S. oryzae*. Keim and Webster (7) reported a fourfold increase in number of propagules when sclerotia were incubated with bits of noninfected rice straw in moist, autoclaved soil in petri dishes. This paper expands on their work and reports on the competitive saprophytic ability of *S. oryzae* derived from sclerotia.

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MATERIALS AND METHODS

Isolate D-30 of *S. oryzae* which produces abundant sclerotia in culture and which is highly virulent (2) was used in the preparation of radioactive sclerotia. Three ml of sterile, molten asparagine-glucose agar (AGA) with 5 μCi D-glucose-^{14}C (U)/ml (specific activity = 2.79 mCi/mmole, New England Nuclear, Boston, MA 02118) were pipetted into a sterile petri dish. A 5-cm-square piece of boiled, sterilized cellophane was placed over the warm, liquid medium in the dish. The cellophane spread the medium out into a thin 5-cm-square layer. The agar was allowed to cool, the fungus was transferred to the center of the cellophane, and the dishes then were incubated in a closed plastic box with moist paper towels placed in the bottom to prevent premature drying of the medium. Each

petri dish yielded about 1,000 labeled sclerotia. Germination tests on 2.0% water agar using 200 radioactive sclerotia indicated they were 92% viable. Counts per minute (cpm) of single sclerotia were obtained by placing a whole sclerotium in a scintillation vial with 5 ml of toluene cocktail (6 g PPO: 2,5-diphenyloxazole and 0.1 g POPOP : 1,4-bis-[2-(5-phenyloxazolyl)]-benzene dissolved in 1 liter of toluene and counting for 5 min in a Beckman LS 233 liquid scintillation spectrometer.

Along with sand, Stockton clay adobe soil from two locations on which rice had been grown for a number of years, and which was naturally infested with *S. oryzae*, was used for the study. The soils had two widely differing concentrations of natural inoculum and were compared for differences in the saprophytic colonization ability of *S. oryzae*. Wet sieving (8) revealed that the soil designated A contained 1.0 naturally occurring sclerotium per gram and that the soil designated B contained 30.8 naturally occurring sclerotia per gram. Naturally occurring sclerotia were removed from the soil by grinding the soil with a Keystone hand mill and then passing the ground soil through a 39.4-mesh/cm (0.149 mm pore size) screen. The sclerotia were retained on the screen. The rice sheaths used in the study were obtained from healthy, mature rice plants grown in the greenhouse and were used without autoclaving.

Radioactive sclerotia were introduced into soil by adding 30 g of air-dry, screened soil to a sterile petri dish, sprinkling 20 radioactive sclerotia on the surface of the soil and then covering the sclerotia with 30 g more of the screened soil. In the treatments in which dried rice sheaths were added to soil, 30 g of screened soil was placed in the petri dish and five, 2-cm lengths of the dried rice sheaths were placed on the soil with the concave side up. Four radioactive sclerotia were placed directly on top of each piece of rice sheath and 30 g more screened soil was spread over the sheaths and sclerotia.

Sterile distilled water was pipetted onto the surface of the soil at the beginning of the experiments and once a week the petri dishes were weighed and more water was added to compensate for that lost by evaporation. Samples of soil were taken after moisture equilibration had occurred to determine water potential (ψ) at that time. Water potential was determined using a thermocouple psychrometer (1). Soil was autoclaved at 125 C for 90 min in 400-ml beakers. Eight treatments of each soil included autoclaved and nonautoclaved soil at -2.3 and -0.9 bars, with and without rice sheaths. There were five replications of each treatment and all plates were incubated at room temperature for 60 days. At the end of this period, all sclerotia were extracted (8), counted, and individually placed in vials for the scintillation counts.

In another experiment, five nylon mesh bags (pore size = 0.2 mm) were each filled with 50 g of air-dry Yolo fine sandy loam soil containing 100 radiolabeled sclerotia of *S. oryzae* and 200 mg chopped, healthy rice sheaths. The bags were sealed shut with a hot soldering iron and buried 12 cm below the soil surface in the field where the soil used to fill the bags was obtained. The field had not previously been cropped to rice and naturally occurring sclerotia of *S. oryzae* were not detected in the soil by the wet-sieving technique (8). The field had been sprinkler-irrigated 1 wk before the bags were buried and was sprinkler-irrigated once a week for 5 wk after burial of the bags. Approximately 3 cm of water were applied to the field at each irrigation. The soil temperature during the afternoon at the 12-cm depth was 22-25 C for the 5-wk period (May-June, 1977). At the end of 5 wk the bags were removed from the soil and all sclerotia were extracted (8), counted, and individually placed in vials for the scintillation counts.

RESULTS

A single radioactive sclerotium was placed on each of five water agar and three AGA plates. When colonies derived from the germinated, radiolabeled sclerotia had produced four to 10 sclerotia, all propagules were transferred individually to scintillation vials and the cpm of the sclerotia was determined. In all cases, new sclerotia were not radioactively labeled (background cpm) and were distinguishable from the germinated, radiolabeled sclerotia (Table 1). As a result of these data any new sclerotia produced saprophytically in the soil incubation experiments were assumed to be nonlabeled and therefore distinguishable from the labeled sclerotia originally introduced into the dishes. The cpm of 100 labeled sclerotia before introduction into soil and of 100 labeled sclerotia after 60 days incubation in soil where rice sheaths were not added were essentially the same (Fig. 1). The cpm of labeled sclerotia followed a semi-logarithmic distribution which may have been due to differences in sclerotium size, carbon dioxide fixation by *S. oryzae*, or the use of the cellophane as a carbon source by *S. oryzae* for formation of some of the sclerotia. All sclerotia extracted from soil after the 60-day incubation period were segregated into two groups. Sclerotia with less than 20 cpm (background) were considered to be new sclerotia produced as a result of saprophytic colonization during the incubation period (Fig. 2). Sclerotia with cpm greater than 20 were considered to be labeled sclerotia which were originally introduced into the soil (Fig. 1). For each treatment, the data from the five replications were averaged and are presented in Table 2.

There was no apparent difference between the production of sclerotia by *S. oryzae* in the soil naturally infested with few sclerotia (Table 2, soil A) and the production of sclerotia in the soil naturally infested with many sclerotia (Table 2, soil B). Differences in natural sclerotia populations between the two thus do not reflect a greater ability of *S. oryzae* to colonize organic matter in one soil as opposed to the other soil. In fact, the sand used as an incubation medium produced results similar to the soils from rice fields. The two water potentials also were not different in effect upon production of sclerotia in soil. Combining the results of the A and B soils revealed that the percentage recovery of the introduced sclerotia was 77.2 for the treatments with no rice sheaths and only 46.5% for the treatments with rice sheaths (Table 2).

In treatments where rice sheaths were not added only labeled sclerotia were recovered (Table 2). In both the autoclaved and nonautoclaved soil *S. oryzae* was not able to use residual organic material present in the soil to produce more sclerotia. Either low inoculum potential or low quantity or quality of organic material in these treatments resulted in lack of saprophytism by *S. oryzae*.

Hudson (4) has shown that leaves and stems of plants are colonized by various parasitic and saprophytic microorganisms before they fall to the ground. The rice sheaths used in this study probably contained other organisms which may have had an effect on the ability of *S. oryzae* to colonize the sheaths. *Sclerotium oryzae* could at least partially overcome competition from prior colonists since sclerotia placed on top of nonsterilized rice sheaths in autoclaved soil germinated, and the fungus colonized the sheaths and increased the number of sclerotia (Table 2). In nonautoclaved soil the saprophytic ability of *S. oryzae* was considerably less. A few of the sclerotia germinated, the fungus colonized the sheaths, and produced several additional sclerotia (Table 2). However, there was no net increase in the numbers of recoverable sclerotia per gram of soil.

Only radioactive sclerotia of *S. oryzae* were recovered from soil in nylon mesh bags buried in the field for 5 wk. The average percentage recovery of radioactive sclerotia from the five bags was 92.8. In the field *S. oryzae* was not able to use either the chopped rice sheaths added to the soil or naturally occurring organic material present in the soil to produce sclerotia.

DISCUSSION

According to Griffin (3) the competitive saprophytic

TABLE 1. The counts per minute (cpm) of radioactive sclerotia of *Sclerotium oryzae* germinated on agar and secondary sclerotia produced in colonies derived from radioactive sclerotia

Agar medium[a]	Germinated radioactive sclerotia[b]	Counts/min of secondary sclerotia[c]
Asparagine-glucose	759.4	7.8[d], 6.2, 6.6, 5.6, 7.0, 8.0, 6.8, 6.2, 8.2, 7.0
	233.8	7.0, 8.0, 7.8, 7.0, 8.0, 9.8, 7.6
	67.2	8.8, 5.8, 7.4, 7.0, 6.8, 7.2, 6.4, 7.2
Water	222.0	5.6, 5.6, 4.8, 4.6, 5.8, 5.2
	37.4	6.8, 6.8, 6.0, 7.8, 5.8
	164.4	8.2, 6.8, 6.4, 6.0
	56.2	6.2, 5.6, 5.2, 5.8, 6.2, 8.2
	160.2	6.2, 5.6, 5.2, 6.4

[a]Medium used for germination of radioactive sclerotia and for growth of *S. oryzae* mycelium derived from radioactive sclerotia.
[b]A single radioactive sclerotium placed on agar and removed after the resulting colony of *S. oryzae* had produced four to 10 additional sclerotia.
[c]Sclerotia of *S. oryzae* produced on agar in a colony derived from a single, germinated, radioactive sclerotium.
[d]Background counts/min = 7.0 ± 4.0.

TABLE 2. Effect of soil water potential, addition of rice sheaths to soil, and autoclaving soil on the recovery of labeled sclerotia and production of new sclerotia of *Sclerotium oryzae*

Treatment	^{14}C-Labeled sclerotia recovered[a] from a soil with a moisture of:		Nonlabeled sclerotia recovered[b] from a soil with a moisture of:	
	−2.3 bars (no.)	−0.9 bars (no.)	−2.3 bars (no.)	−0.9 bars (no.)
Nonautoclaved sand				
rice sheaths	12.6[d]	16.6	3.6	0.6
no rice sheaths	14.6	16.0	0	0
Autoclaved sand				
rice sheaths	10.6	10.0	12.0	48.6
no rice sheaths	17.0	17.6	0	0
Nonautoclaved soil A[c]				
rice sheaths	10.0	9.4	1.4	0
no rice sheaths	13.2	15.0	0.2	0
Autoclaved soil A				
rice sheaths	8.2	10.4	38.6	28.8
no rice sheaths	16.6	17.0	0	0
Nonautoclaved soil B[c]				
rice sheaths	12.2	7.2	1.6	2.6
no rice sheaths	15.8	11.8	0	0
Autoclaved soil B				
rice sheaths	8.4	8.6	15.2	30.0
no rice sheaths	18.0	16.2	0	0

[a]Initially 20 labeled sclerotia of *S. oryzae* introduced in each replication and incubated 60 days.
[b]Sclerotia produced as a result of saprophytic colonization of dead organic material by *S. oryzae*.
[c]A and B refers respectively to soil from fields cropped many years to rice and naturally infested with few and many sclerotia of *S. oryzae*. Naturally occurring sclerotia were removed by sieving before the soil was used.
[d]All values are means of five replications.

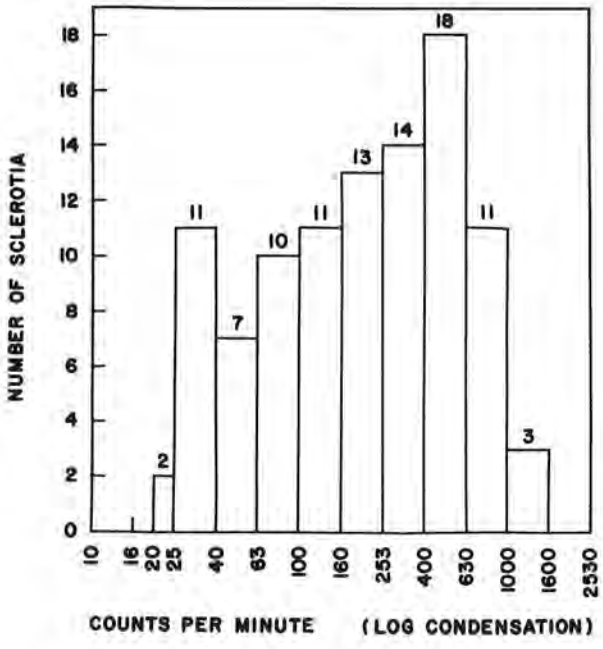

Fig. 1. Histogram of counts per minute (cpm) of 100 radioactive sclerotia of *Sclerotium oryzae* recovered after incubation for 60 days in soil without rice sheaths added. The counts/min of 100 radioactive sclerotia of *S. oryzae* before introduction into soil produced a similar histogram.

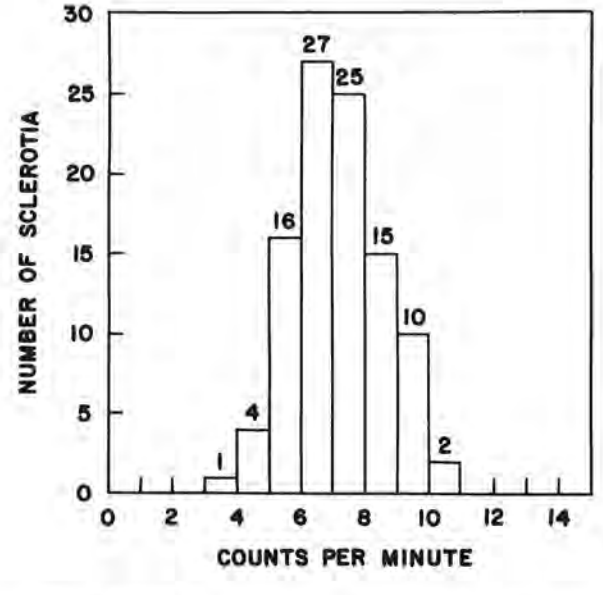

Fig. 2. Histogram of counts per minute (cpm) of 100 nonradiolabeled sclerotia of *Sclerotium oryzae* extracted from soil which were produced as a result of colonization of rice sheaths by germinated, radioactive sclerotia (background cpm = 7.0 ± 4.0). The cpm of 100 sclerotia of *S. oryzae* extracted from naturally infested soil produced a similar histogram.

colonization of a substrate by a fungus is determined by the innate competitive saprophytic ability of that fungus, its inoculum potential, and environmental conditions. Since colonization of rice sheaths occurred in the autoclaved soil, it appears that the environmental conditions used in this study were favorable for *S. oryzae* to colonize organic material. Also, since the radioactive sclerotia were placed directly on top of rice sheaths and were 92% viable, inoculum potential should not have been a factor in those treatments. Griffin (3) further states that poor saprophytic colonization of a soilborne substrate by a given fungus may depend upon competition and antibiosis. These factors may play a role in nonautoclaved soil since *S. oryzae* apparently cannot compete well with the natural soil microflora for the rice sheath substrate. Poor saprophytic colonization of the rice sheaths in nonsterile soil is probably due to fungistasis of sclerotia of *S. oryzae* as reported by Keim and Webster (6, 7).

Even though two different soil types were used, the field and the laboratory data indicate that the competitive saprophytic ability of *S. oryzae* derived from sclerotia is limited in nonsterile soil. We conclude that colonization of healthy rice residue by mycelium from sclerotia of *S. oryzae* is not an important avenue for production of new sclerotia. Therefore, rice tissue parasitically infected by *S. oryzae* remains the most important source of sclerotia.

While monitoring fungal propagule populations in the soil it is important to know if multiplication and turnover of propagules is occurring. In this study the use of radioactive sclerotia conclusively showed whether a propagule recovered after a period of time was one of the originally introduced sclerotia or one produced in the soil environment. We believe this technique could be applied to a variety of fungi as an aid to following fungal propagule populations in soil.

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