The Relationship of Sporulation, Sclerotia Production, and Growth Rate to Virulence and Fitness of Sclerotium oryzae

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ABSTRACT

Attributes assumed likely to contribute to the relative success or fitness of *S. oryzae* were studied in 20 isolates. Quantitative differences in the rate and amount of conidia and sclerotia produced and growth rate did not correlate with an apparent lack of fitness among isolates extreme for virulence, or with an observed skewed distribution in nature of isolates carrying the "A" mating type allele. Production of conidia and sclerotia and growth rate are probably inherited independently of each other, virulence, and mating type since no relationships between these characters were revealed with regression and correlation analysis.

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Stem rot of rice (*Oryza sativa* L.) is caused by *Magnaporthe salvinii* (Cattaneo) Krause and Webster (3, 5). The fungus is best known for its sclerotial state, *Sclerotium oryzae* Catt. The conidial state has been referred to as *Nakatea sigmoidea* (Cav.) Hara, *Vakrabeeja sigmoidea* (Cav.) Sub., or *Helminthosporium sigmoideum* Cav. The fungus is simply heterothallic (6); two alleles at one locus determine the compatible mating reaction for the ascomycetous state.

It is useful to know if a relationship between virulence and fitness (as indicated by relative abundance or survival ability) exists in a pathogen since it may influence the effectiveness of resistance against that pathogen in its host. If highly virulent isolates of a pathogen are highly fit, it is less likely that resistance will be an effective control method. Van der Plank (11) has proposed that highly virulent races of a pathogen will be less fit because of the influence of stabilizing selection when horizontal-type resistance occurs in the host. Since stem rot resistance is quantitatively inherited (3), it seems that stem rot resistance in rice is the type of resistance with which van der Plank was concerned when he coined the term "horizontal resistance". An understanding of the relationship between virulence and fitness in *S. oryzae* would be useful in determining if highly virulent and fit races might predominate in a natural population of this pathogen and its subsequent implications regarding potential development of resistant cultivars.

An indication that isolates of *S. oryzae* differ in fitness was apparent when a random sample of 69 isolates was characterized for virulence. The most- and least-virulent isolates were not isolated as frequently as those of intermediate virulence (2). The hypothesis that isolates of intermediate virulence are better fit seems logical since these isolates were more frequent in the sample studied.

If alleles of a particular locus have no selective advantage or disadvantage, an equal distribution of these might be expected in a sample from the natural population. Determination of mating type for over 650 isolates of *S. oryzae* obtained from over 200 fields representing the rice-growing regions of California revealed that the mating type alleles (A,a) were distributed in an 8:3 rather than a 1:1 ratio, indicating that isolates carrying the A allele may be better fit. Further study indicated that the 8:3 distribution of mating type alleles observed was not due to sampling technique or error (R. K. Webster and S. A. Ferreira, *unpublished*).

For these reasons, several characters of *S. oryzae* were studied to determine their possible contribution to fitness in *S. oryzae*. The attributes studied were: (i) the number of conidia produced and rate of conidia production, (ii) the number of sclerotia produced, and (iii) the mycelial growth rate. Selection of these traits was based on the following rationale. Although the role of conidia in stem rot disease is not well understood at present, it is reasonable to suggest that, as with many plant diseases (1, 9, 10, 11), conidia may function as secondary inoculum in dispersing the pathogen in the field. Conidia have been observed on stem rot lesions approximately 60 days after infection in the field. It appears (R. K. Webster, *unpublished*) that the amount of infection increases during the season when no additional primary inoculum propagules (sclerotia) are being produced, suggesting that conidia may function in disease spread from initial foci of infection. In addition, inoculations of Colusa rice seedlings in the laboratory with conidial suspensions indicated that hyphae from conidia can initiate infection and cause symptoms identical to those caused by sclerotial inoculations. Therefore, the ability to quickly produce conidia, or to produce conidia in large numbers, may be important in determining the fitness or the success of individual isolates of *S. oryzae*. Isolates producing conidia more rapidly or abundantly than others may infect more plants. These infections when mature would have sclerotia produced in them which serve as primary inoculum the following year. Consequently, a greater proportion of the overwintering inoculum would be expected to be derived from isolates of the pathogen with greater capacities for sporulation. Given many cycles of disease, these isolates would tend to predominate. Similar arguments can be used to consider sclerotial production and mycelial growth rate as attributes which might contribute to the fitness of *S. oryzae*.

Quantitative estimates of these traits were determined for isolates of *S. oryzae* differing in mating type and virulence to determine if differences in fitness might account for the observed frequencies of the mating type alleles in nature, and to obtain insight into the epidemiology of stem rot of rice.

MATERIALS AND METHODS.—*Isolates of S. oryzae.*—Twenty single-spore isolates, each obtained from a separate stem rot-infected Colusa or Calrose plant from four widely separated fields in Butte County in California, were included in the present study. They represent both mating types and a range of virulence as determined by the seedling test described previously (2).

Sporulation studies.—To measure sporulation ability, 15 mg of sclerotia from each isolate were floated on 30 ml

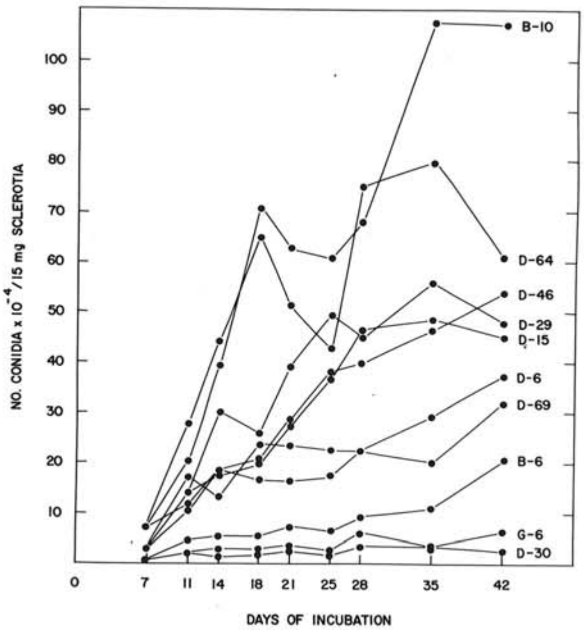

Fig. 1. Conidia production by 10 "A" (mating type) isolates of *Sclerotium oryzae* differing in virulence.

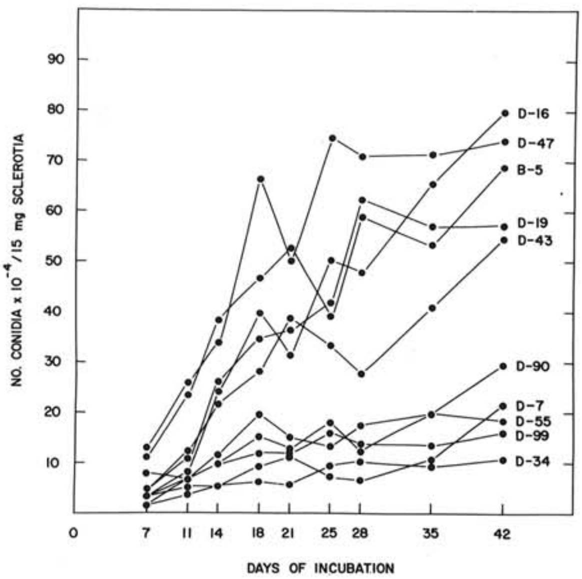

Fig. 2. Conidia production by 10 "a" (mating type) isolates of *Sclerotium oryzae* differing in virulence.

TABLE 1. A comparison of selected fitness attributes among 20 isolates of *Sclerotium oryzae*

Isolate	MT[a]	$LR_{1/2}$[b]	$KR_{1/2}$[c]	SR[d]	SC[e]	SP[f]	GR[g]
D-30	A	2	5	0.13	3.3	21	0.86
B-6	A	4	9	0.38	14.2	14	1.14
G-6	A	3	6	0.16	5.2	18	0.97
D-46	A	3	6	2.00	52.6	...	0.79
D-6	A	5	15	1.62	30.4	25	0.80
D-64	A	3	14	5.25	77.1	...	1.22
D-29	A	5	13	2.40	56.5	...	0.69
B-10	A	5	16	5.83	103.8	17	0.90
D-69	A	7	22+	1.82	28.6	...	0.81
D-15	A	5	18	1.59	48.2	15	0.81
D-43	a	3	8	2.38	48.1	16	0.70
B-5	a	4	12	3.03	65.9	...	0.87
D-7	a	4	15	0.62	15.5	21	0.87
D-55	a	4	15	0.91	22.1	23	0.86
D-47	a	4	15	4.71	82.6	19	0.87
D-34	a	6	22+	0.46	10.8	9	0.79
D-99	a	6	20	0.73	16.9	...	0.73
D-19	a	6	22	2.46	62.8	20	0.81
D-16	a	4	10	2.27	71.1	...	0.84
D-90	a	5	15	0.79	23.7	32	0.77

[a]MT = mating type.
[b]$LR_{1/2}$ = time in days for half of 10 inoculated seedlings to be infected.
[c]$KR_{1/2}$ = time in days for half of 10 inoculated seedlings to be killed.
[d]SR = sporulation rate (number of conidia $\times 10^4$ per day per 15 mg sclerotia).
[e]SC = sporulation capacity (number of conidia $\times 10^4$ per 15 mg sclerotia).
[f]SP = sclerotia production (mean number of sclerotia per microscope field in cornmeal agar culture).
[g]GR = growth rate (mm growth per hour).

TABLE 2. Sclerotia production of 19 isolates of *Sclerotium oryzae* differing in mating type and virulence

Isolate	MT[b]	$LR_{1/2}$[c]	$KR_{1/2}$[d]	Number of sclerotia produced after various times (days) of incubation[a] 9	14	24	28
C-6	A	3	22+	0	19	10	14
B-6	A	4	9	0	14	11	14
B-10	A	5	16	0	14	15	17
G-1	A	5	20	4	21	31	21
G-6	A	3	6	21	12	13	18
G-22	A	3	9	14	16	15	19
D-6	A	5	15	18	23	18	25
D-13	A	4	13	0	9	6	9
D-15	A	5	18	12	16	18	15
D-30	A	2	5	17	18	18	21
D-7	a	4	15	16	21	24	21
D-10	a	4	22+	10	13	11	16
D-19	a	6	22	22	21	22	20
D-34	a	6	22+	10	10	9	9
D-43	a	3	8	16	19	17	16
D-47	a	4	15	17	22	19	19
D-55	a	4	15	17	24	20	23
D-90	a	5	15	27	38	35	32
D-98	a	4	15	0	10	8	9

[a]Sclerotia production is indicated as the mean number of sclerotia per microscope field in cornmeal agar culture.
[b]MT = mating type.
[c]$LR_{1/2}$ = time in days for half of 10 inoculated 25-day-old Colusa seedlings to be infected.
[d]$KR_{1/2}$ = time in days for half of 10 inoculated 25-day-old Colusa seedlings to be killed.

distilled water (4). There, sclerotia germinated and produced conidia which were harvested from petri dishes as a conidial suspension. Three drops of a wetting agent, sodium 2-ethylhexyl sulfate (Tergitol Anionic 08), were added, and the conidial suspension was stirred for 3 minutes with a magnetic stirrer. Aliquots of the suspension were counted with a haemocytometer and an estimate was made of the number of conidia produced by each isolate based on total volume of the suspension from two petri dishes. Conidial counts were made twice a week for 42 days.

The rate of conidia production (sporulation rate) was calculated statistically by fitting a first order linear regression model to the linear interval of data. The parameter b (regression coefficient) for each isolate was considered to be the statistical measure of the sporulation rate for the isolate. Similarly, a statistical measure of the sporulation capacity (total conidia produced after 42 days

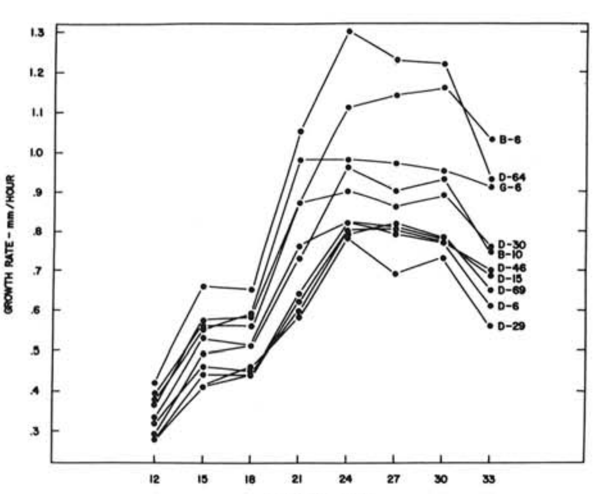

Fig. 3. Growth rate at different temperatures of 10 "A" (mating type) isolates of *Sclerotium oryzae* differing in virulence.

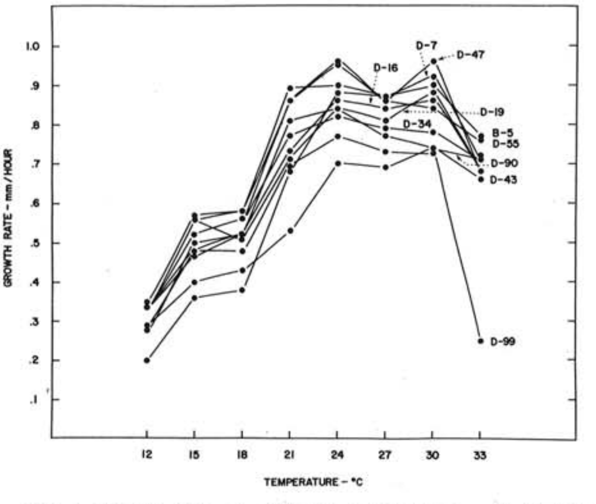

Fig. 4. Growth rate at different temperatures of 10 "a" (mating type) isolates of *Sclerotium oryzae* differing in virulence.

incubation) was determined by fitting a second order linear regression model to the data.

Sclerotia production.—In order to measure the sclerotia-producing capacity of *S. oryzae,* agar plugs of the different isolates were inoculated to Difco cornmeal agar (CMA) plates. When sclerotial initials were evident, random fields under a dissecting microscope were picked, and the sclerotia counted. The average number of sclerotia per microscope field was used as the measure of the ability of an isolate to produce sclerotia.

Growth rate.—In order to determine the growth rates of *S. oryzae,* growth was observed on CMA plates inoculated with agar plugs of the isolates at 12, 15, 18, 21, 24, 27, 30, and 33 C. After colony diameters were measured at 24-hour intervals, growth rates for each isolate were calculated from a first order linear regression model statistically fitted to the data. The value of b (regression coefficient) was used as the estimate of the growth rate of each isolate.

RESULTS.—*Sporulation in S. oryzae.*—The results of studies to determine the relative ability of 20 isolates of *S. oryzae* to produce conidia rapidly or abundantly are given in Fig. 1 and 2 and Table 1. The data show that the rate of conidia production and the final number of conidia produced are highly variable characters.

Sclerotia production in S. oryzae.—The observed sclerotia production by the isolates studied is given in Tables 1 and 2. The data show that sclerotia production in *S. oryzae* is not a highly variable character, since most isolates produced about the same number of sclerotia at about the same rate.

Growth rate in S. oryzae.—The results of studies on the growth of *S. oryzae* are given in Fig. 3 and 4 and Table 1. The growth rate is a highly variable character among isolates. Although the optimum temperature for growth for most isolates was 27 C, some isolates grew optimally at higher or lower temperatures.

Mating-type allele distribution, virulence, and selected fitness traits of S. oryzae.—In an analysis of variance with

TABLE 3. Analysis of variance indicating the relationship between mating type, virulence, and selected fitness traits in *Sclerotium oryzae*. The quantitative measure of each trait was based on a mean of 10 isolates of *Sclerotium oryzae*

Trait	Quantitative measure of trait for mating type:		
	A	a	F
Lesion initiation ability ($LR_{1/2}$)	4.2[a]	4.6	0.48
Killing ability ($KR_{1/2}$)	11.3[b]	14.7	2.30
Sporulation rate	2.12[c]	1.84	0.14
Sporulation capacity	42.0[d]	42.0	0.01
Sclerotia production	17.3[e]	17.0	0.01
Growth rate	0.90[f]	0.81	2.47
F ($P = .05$) = 4.41			
F ($P = .01$) = 8.28			

[a]Time in days for half of 10 inoculated seedlings to be infected.
[b]Time in days for half of 10 inoculated seedlings to be killed.
[c]Number of conidia $\times 10^4$ per day per 15 mg sclerotia.
[d]Number of conidia $\times 10^4$ per 15 mg sclerotia.
[e]Mean number of sclerotia per microscope field in cornmeal agar culture.
[f]mm growth per hour.

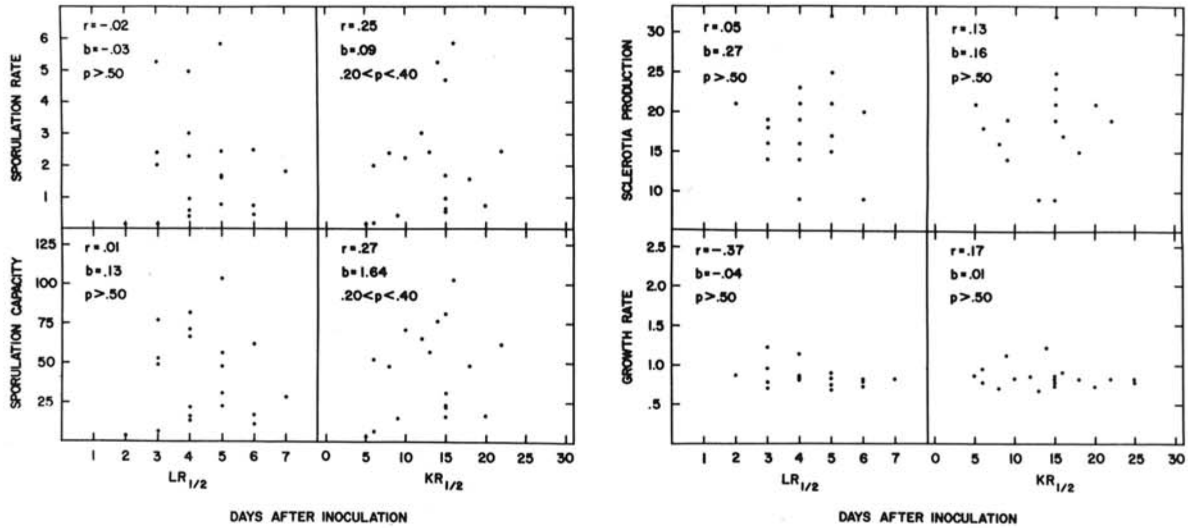

Fig. 5. Correlation and regression relationships between four fitness traits and virulence for 20 isolates of *Sclerotium oryzae*. Virulence was estimated by the time for half of 10 inoculated 25-day-old rice seedlings (cultivar Colusa) to be infected (LR 1/2) or killed (KR 1/2).

mating types as treatments (Table 3), no significant differences in virulence, sporulation rate, sporulation capacity, sclerotia production, or growth rate were associated with isolates of the fungus carrying a particular mating-type allele. Therefore, differences in virulence or the fitness attributes studied do not appear to account for the disparity in distribution of mating-type alleles in nature among isolates of *S. oryzae*.

Fitness and virulence in S. oryzae.—The results of correlation and regression analyses between the fitness parameters (sporulation rate, sporulation capacity, sclerotia production, growth rate) and virulence of *S. oryzae* as measured by the ability of isolates to initiate lesions or kill Colusa rice seedlings (2) are given in Fig. 5 and Table 1. No simple direct relationships between the assumed fitness attributes and virulence were suggested. However, the most virulent isolates (D-30, G-6, and B-6) had the lowest sporulation rates and capacities (Table 1).

Relationships among the fitness parameters of S. oryzae.—The results of correlation and regression analyses among the fitness attributes studied are given in Fig. 6, 7, and 8. A relationship between sporulation rate and capacity was found to exist in *S. oryzae*. Except for this relationship, the data show that there were no relationships among the various assumed fitness parameters. This suggests that these attributes are independent of each other and that it is chance alone that determines which become associated in individual isolates.

DISCUSSION.—The predominance of one mating-type allele in a natural population of *S. oryzae* could not be explained on the basis of a relationship between mating type and virulence, sporulation rate or capacity, sclerotia production, or growth rate. Study of additional

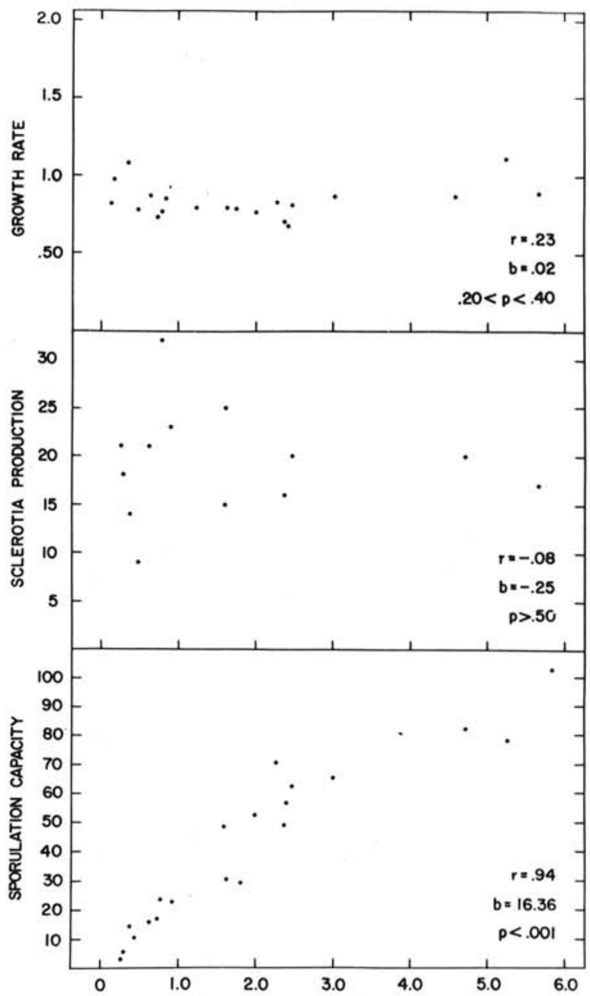

Fig. 6. Correlation and regression of growth rate, sclerotial production, and sporulation capacity on sporulation rate for 20 isolates of *Sclerotium oryzae*.

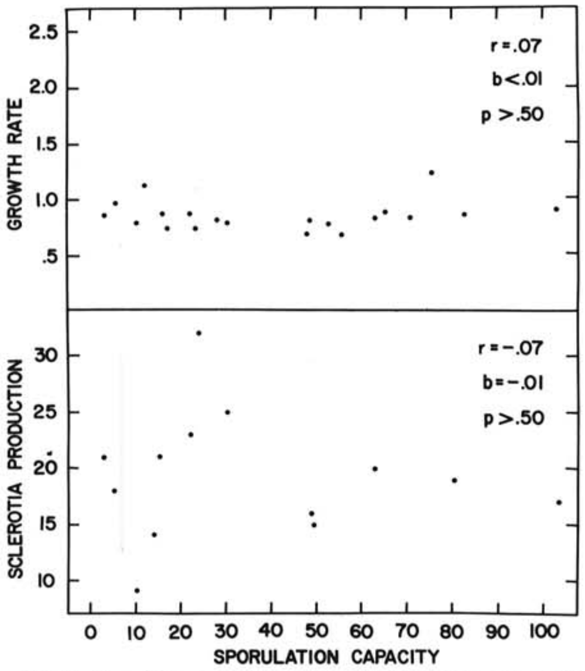

Fig. 7. Correlation and regression of growth rate and sclerotial production on sporulation capacity for 20 isolates of *Sclerotium oryzae*.

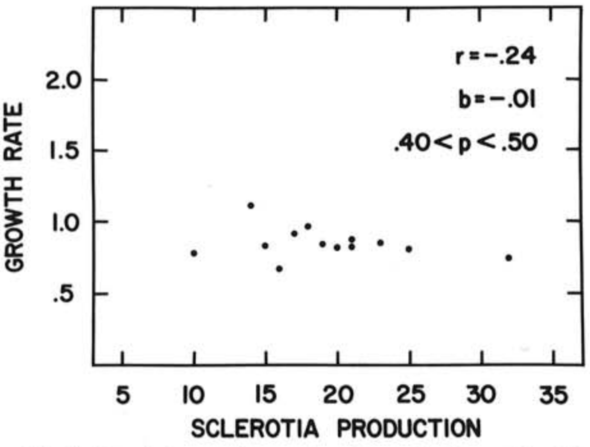

Fig. 8. Correlation and regression of growth rate on sclerotial production for 20 isolates of *Sclerotium oryzae*.

traits to determine the basis for the apparent increased fitness associated with one mating-type allele is needed.

Stem rot of rice has been characterized by many authors (7, 9) as a late-season disease. Webster (*unpublished*) observed the greatest increase in disease severity occurred late in the season as rice approaches maturity. Characteristically, numerous sclerotia are formed on infected tissues (1, 5, 6, 7, 8) and Keim (4) showed that sclerotia were formed on rice residues in soil if the residue had been infected with *S. oryzae* before it was incorporated in rice soils. The ability to produce sclerotia, then, appears to play an important role in the biology of *S. oryzae*. Perhaps, because it may be important to *S. oryzae,* differences in sclerotia production would not explain the relative abundance of isolates differing in mating type or virulence. However, if rice tissues or straw had been the substrate on which sclerotia production had been studied, the natural capacity for isolates to produce sclerotia may have been reflected more clearly, and perhaps a relationship between abundance of isolates and sclerotia production might have been found.

Since the fitness attributes studied all appear to be independent (except for sporulation rate and capacity), it is likely that the relative success of isolates depends on how the various traits are distributed by chance to an individual isolate. If some fitness traits are more important than others, they would determine the overall fitness of individual isolates. This may be the case for sporulation and virulence in *S. oryzae*.

The most virulent isolates included in the present study were found to be low in sporulation abilities suggesting that rate and capacity for sporulation may account at least in part for the lower frequency of highly virulent isolates observed in the sample studied. Sporulation rates and capacities were measured from germinating sclerotia on distilled water. Results were thought to be similar to sclerotia germinating on paddy water. Perhaps the relationship between sporulation and virulence would be more clear if sporulation were measured from infected seedlings or stem pieces, since this would more closely reflect the ability of isolates of *S. oryzae* to spread secondarily in nature.

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