Achlya klebsiana and Pythium species as Primary Causes of Seed Rot and Seedling Disease of Rice in California

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Accepted for publication 15 January 1970.

ABSTRACT

Within a few days after seeding, water-sown rice often is severely infected by seed-rot and seedling disease resulting in reduced stands. The disease, although prevalent throughout the rice-producing areas of California, is generally more severe when temp are cool and unfavorable for the growth of rice. *Achlya klebsiana* or *Pythium* species were usually isolated from decayed seeds and infected seedlings. Outgrowths of whitish hyphae characteristically radiated from cracks in the glumes or from the collar of the plumule. The standard California varieties (Caloro, Colusa, and Calrose) were equally susceptible. Isolates of *A. klebsiana* did not differ significantly in pathogenicity. Optimum temp for growth of *A. klebsiana* isolates in culture was 27-30 C, but pathogenicity did not differ significantly between 20-30 C (a mean of 52.8% of the plants became diseased over this range). *Pythium* isolates grew well in culture at 30 C, but were significantly less pathogenic (17.2% plants infected) than at 25 C (48.6% plants infected) or 20 C (95.5% plants infected). Although these results are consistent with field observations, cool temp apparently increase disease severity primarily through an adverse effect on growth of rice seedlings rather than by favoring growth of the pathogen. Phytopathology 60:964-968.

A major problem in rice production in California is the establishment of optimum and uniform stands. Ungerminated seeds and diseased seedlings are usually infected with various fungi. The majority of the fungi on rotted seed or dying seedlings are members of either the *Saprolegniaceae* or *Pythiaceae*. The possibility that fungi belonging to these groups may be involved in a rice seedling disease complex has been recognized for many years (3, 4, 5, 6, 10, 12), particularly in early-planted rice seed beds or nursery beds in Taiwan and Japan (9). Convincing demonstration of the pathogenicity of these organisms is lacking, however.

In general, workers (1, 2, 6) have agreed that seed rot and damping-off of rice is most serious in seasons when temp during the seedling stage are low and the development of seedlings is delayed or uneven. Wei et al. (12) considered seed-rot and damping-off primarily as a physiological disorder in which seedlings weakened by low temp or poor aeration effects are more readily attacked by water molds and other fungi.

Although most reports agree that various species of water molds are the main cause of this disease, there is little agreement on which species are involved or which are the most important. For example, Ito (5) reported that *Pythium aphanidermatum* and *Pythium helicum* were strongly pathogenic to rice in petri-dish cultures, but other *Pythium* species were only moderately so. He stated that *Achlya* species were only slightly pathogenic, but suggested that they may have been more destructive than observed in his tests if the seedlings had been grown at lower temp. Thus he concluded that the rot disease of rice seedlings is due principally to *Pythium* species. Sawada (10), however, considered *Achlya prolifera* to be the cause of this disease. Hemme & Abe (4) also referred to *A. prolifera* as the sole cause. Ito & Nagai (6) isolated twelve species of fungi from rice seedlings from many different rice-growing centers in Japan, but not *A. prolifera*. Besides several *Pythium* species, they isolated *A. americana, A. flagellata, A. flagellata* var. *yezoensis, A. megasperma,* and *A. oryzae*. They concluded from pathogenicity studies that *A. americana* caused only slight injury, whereas the others caused severe damage. In addition to *Achlya* and *Pythium* species, Hashioka (3) isolated *Brachysporium, Fusarium,* and *Penicillium* species from both infected seeds and soil. In laboratory tests, *Achlya* was pathogenic to growing seedlings on both agar media and sterilized soil, but when added to the soil under glass it was not pathogenic. When plants were chilled in the refrigerator for 10 hr at 1 C before inoculation, severe infection often resulted, however. Lamey (8) reported that seed-rot and damping-off result in slight to severe stand reduction in rice in Louisiana, depending on local conditions, but the cause was not stated.

The seed-rot and seedling disease of water-sown rice that occurs in California rice fields has not been previously investigated. This study was initiated to determine the role of *Achlya klebsiana* and *Pythium* sp. in these diseases. The effect of temp on the pathogens, disease development, and varietal responses were also considered. A preliminary report has been published (11).

MATERIALS AND METHODS.—*Source and maintenance of isolates.*—Isolates were obtained from rotted seed or diseased seedlings collected from fields near Robbins, Biggs, and Colusa, Calif. All *Achlya* stock cultures originated as single zoospores from the original isolations and were maintained on potato-dextrose agar (PDA). Stock cultures of *Pythium* isolates, established from single hyphal tips, were also maintained on PDA.

Production of inoculum.—To obtain zoospore suspensions for use as inoculum, discs cut from the advancing margin of *Achlya* colonies on PDA were placed in plates of sterile distilled water. Twenty-four hr later, the zoospores and water from the plates were consolidated and the spore density determined by haemocytometer counts.

Pathogenicity tests.—Pathogenicity tests were carried out as follows: Dry field soil from the Rice Experiment

Station, Biggs, California, was screened, moistened, and sterilized for 3 hr at 15-lb. pressure and 121 C and allowed to stand on the greenhouse bench for 48 hr. After the aeration period, 500 g portions of the sterilized soil were placed in sterile unperforated seedling pots. Distilled water was added to a water depth of 5 cm above the soil surface, and was maintained as closely as possible at this level throughout the test period. The seed used throughout was foundation seed of four commercial cultivars: Calrose, Colusa, Caloro, and Earlirose. Before sowing, the seeds were soaked for 24 hr in a 1% solution of sodium hypochlorite and for an additional 24 hr in sterile distilled water. The seed was sown into the water directly onto the surface of the soil with a special template devised to enable seed to be sown separately at a rate per unit area of 16 seeds/pot. Pots were maintained in the greenhouse at 21-27 C under daylength of 14 hr light and 10 hr dark. Inoculum of *Achlya* was added to the flooded pots the same day that seeds were sown. Inoculum of the *Pythium* isolates was prepared by blending mycelium from PDA with sterile distilled water, after which 25-ml portions were pipetted into sterilized field soil prepared as described. The pots were flooded with distilled water and allowed to stand for 3 days before seeds were sown. Pathogenicity tests involving the effect of temp were carried out in Esco water-bath temp tanks.

Growth studies on the pathogen.—Growth rates of the various isolates were determined on both linear growth rate and dry-wt basis. In the first case, growth tubes (dam tubes) containing PDA were inoculated with 4-mm discs cut from advancing margins of colonies produced on PDA. The cultures were incubated in controlled-temp incubators at 6-39 C, with separate sets of cultures at 3-C intervals. Linear growth from the inoculum disc to the edge of the advancing colony was measured at 24-hr intervals. For dry wt determinations, the isolates were grown for 3 days (72 hr) in 125-ml Erlenmeyer flasks containing 50 ml of potato-dextrose broth at the same temp used in the linear-growth study.

RESULTS AND DISCUSSION.—*Symptoms of the disease.*—Seed rot and seedling disease of rice becomes evident within a few days of planting. The most common signs are outgrowths of whitish hyphae from the surface of the seed or collar of the plumule. The hyphae grows from cracks in the glumes, and within a few days a radiating halo of mycelium from the infection point is apparent on the seeds (Fig. 1). The halo consists of coarse bristly mycelium, and often the characteristic sporangia of *Achlya* are easily discernible. The halo of mycelium soon appears green, due to the presence of various algae. In some cases the infected seed appear within a dark circular spot on the soil surface. This, too, may be due to the presence of algae, but is most probably caused by secondary invasion of the seed and fungus by bacteria in the aquatic environment of the seeds and seedlings. If seedlings produce primary leaves and roots before infection by the fungi, they are usually stunted. The leaves and leaf sheaths become discolored and further development is retarded (Fig. 1). Here, as before, the typical halo of mycelium is usually evident.

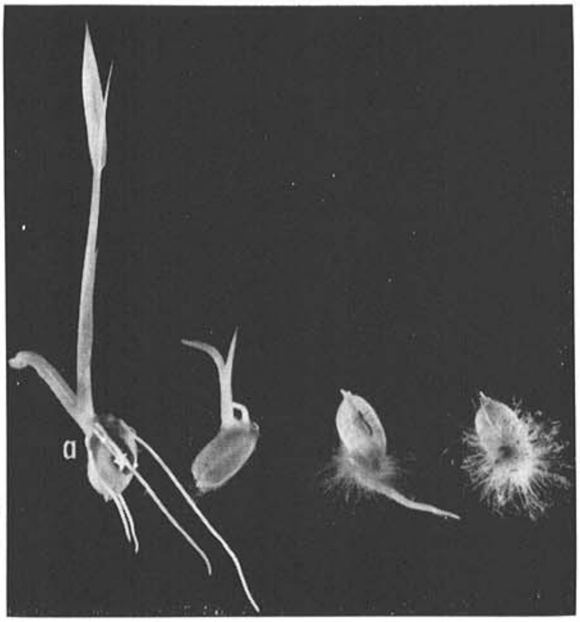

Fig. 1. Rice seed collected from the field 7 days after sowing, showing characteristic outgrowths of hyphae from seed; a = noninfected seedling.

If infection occurs after seedlings are well established, there is generally little apparent effect.

Occurrence of Achlya klebsiana and Pythium sp. on rice seed and seedlings in Calif.—Isolations were made on water agar (15 g agar/liter) from infested seed and diseased seedlings collected from a large number of fields throughout the rice-growing areas of Calif. Seeds and seedlings were placed beneath the agar, and the plates were incubated for 24-48 hr, after which fungi had grown through the agar and the hyphal tips were free from contamination on the surface of the agar. Hyphal-tips were transferred from the water agar onto PDA plates, which were incubated at room temp. The fungi were then identified.

Soil samples collected from the various areas were placed in pots in the greenhouse under 5 cm of standing water, and surface-sterilized seed were sown onto the surface. After 7 to 10 days, isolations were made from infected seeds and seedlings. The following fungi were isolated: *Fusarium roseum* (Link) emend Snyd. & Hans.; *Sclerotium oryzae* Catt.; *Pythium* spp.; and *Achlya* spp. The first two were obtained only a few times, whereas *Achlya* was obtained from over 65% of the diseased seeds and seedlings. *Pythium* species were obtained from about 30% of the diseased seeds and seedlings. In some cases, both *Pythium* and *Achlya* were obtained from the same seeds and seedlings. *Achlya* and *Pythium* isolates were obtained from infected seeds, seedlings, or soil from fields in Fresno, San Joaquin, Yolo, Butte, Glenn, Sutter, and Colusa counties.

Identification of the Achlya isolates.—At least seven species of *Achlya* are reported (3, 4, 5, 10) to cause seedling disease in rice-producing Asian countries, including *Achlya americana, A. flagellata, A. flagellata* var. *yezoensis*, *A. megasperma*, *A. oryzae*, *A. prolifera*, and *A. klebsiana* Pieters. According to Johnson (7), *A. megasperma* should be placed in the subgenus *Subcentrica*, whereas the other species belong to the subgenus *Achlya*. Furthermore, Johnson considered *A. oryzae* merely a form of *A. klebsiana*. He also raised the question as to whether *A. prolifera* as described by Sawada (10) may not have been *A. prolifera*. Since the only practical means for delimiting species in the genus is by comparing the characteristic sexual structures, the isolates were grown on rice or hemp seed that favor development of sexual structures. Rice seed and hemp seed were sterilized in the autoclave for 1 hr; a few seeds were then placed in petri dishes containing sterile distilled water, and a small agar block containing mycelium from an isolate was placed in the plate and incubated in the laboratory for 7 to 14 days. Slide cultures were also made to facilitate observations on the origin of the antheridial elements. Although some variation in structures was observed, the majority of isolates fell within the limits given for *Achlya klebsiana*. As pointed out by Johnson (7), *A. klebsiana* may or may not have pitted oogonial walls under the point of attachment of antheridial cells. Pitting was not prominent in our material, but could be found in each isolate when sufficient material was examined. Although Pieters stated that the antheridial branches of *A. klebsiana* are always diclinous, monoclinous antheridial branches may occur occasionally, as Johnson pointed out. The majority of the antheridial branches observed in our isolates were diclinous, although monoclinous antheridial branches frequently were observed. On the basis of Johnson's monograph (7) of the genus *Achlya*, we concluded that all of our isolates belong to the species *Achlya klebsiana*.

Attempts to identify the *Pythium* isolates thus far have failed. A few spherical sporangia were observed in cultures of both R1-P and R2-P under a variety of culture conditions, but no satisfactory procedure was found for producing the reproductive structures necessary for identification.

Pathogenicity tests.—To determine whether the *Achlya* and *Pythium* isolates are capable of causing seedling disease of rice and are not merely secondary in nature, controlled pathogenicity tests were carried out in the greenhouse. The effect of three isolates of *A. klebsiana* (70-2, Sutter County; 50-2, from Colusa County; and R3-A, from Butte County) on Caloro rice are shown in Table 1. All three isolates were highly pathogenic, respectively causing 52.2, 61.3, and 65.9% diseased plants.

TABLE 1. Pathogenicity of isolates of *Achlya klebsiana* on Caloro rice[a]

Isolate	% Diseased[b]
70-2	52.2
50-2	61.3
R3-A	65.9
Check	0

[a] Based on 2 tests with 4 replications/test per isolate.
[b] % Diseased based on differences in total plants emerged in check and test pots after 15 days.

Pathogenicity of these three isolates was also compared on Caloro, Calrose, Colusa, and Earlirose to determine whether they differ in virulence or whether the current California varieties differ in susceptibility to seedling disease. The conditions were as described for the above test except that inoculum consisted of 60,000 spores/pot. No significant difference (.05) in pathogenicity was observed among the isolates to either Caloro, Colusa, or Earlirose. On Calrose, however, isolate 50-2 was more pathogenic than were isolates 70-2 and R3-A. Earlirose was less susceptible to all the isolates than were the other three varieties, and exhibited stronger seedling vigor throughout the tests. Whether the lower incidence of disease on Earlirose was due to innate resistance or to escape was not ascertained.

Additional tests were made to compare the effect of inoculum level of the three isolates on the four varieties. The incidence of diseased seedlings in pots inoculated with 60,000 or 120,000 spores was about equal. This was probably due to the earlier sporulation of *A. klebsiana* on seeds and organic matter (within 48 hr of inoculation), resulting in a rapid increase of secondary inoculum.

Pathogenicity of Pythium isolates.—Pathogenicity of *Pythium* isolates R1-P and R2-P from Butte County was determined on Caloro rice. The *Pythium* isolates obtained from diseased seeds and seedlings are pathogenic to rice causing disease in 35-72% of the seedlings (Table 3).

Effect of temp on disease severity and pathogen growth.—Early accounts stress the importance of temp on the development and severity of rice seedling disease. One report (12) called it a cold-weather disease, and

TABLE 2. Pathogenicity of three isolates of *Achlya klebsiana* to four rice cultivars

	Cultivars			
Isolate	Caloro	Calrose	Colusa	Earlirose
70-2	61[a]	63	71	39
50-2	60	78	74	45
R3-A	59	61	66	33
Check	9	16	8	5

[a] Total of 6 replications, 16 seeds/replication per test. Numbers indicate diseased seedlings or ungerminated seed 15 days after sowing. Germinability of all four varieties <97%. Inoculum consisted of 6×10^4 300-spores/pot. Earlirose was significantly (5% level) less susceptible to all isolates than were the other varieties.

TABLE 3. Pathogenicity of two *Pythium* isolates to Caloro rice[a]

Isolate	Test no.	% Diseased[b]
R1-P	1	34.8
	2	71.7
R2-P	1	67.3
	2	54.3
Check	1	0
	2	0

[a] Based on a total of 6 replications/isolate per test.
[b] % Diseased based on differences in total healthy plants emerged in check and test pots after 15 days at 25 ± 2 C.

speculated that environment alone may be the cause. The effect of temp on both linear growth and mycelial dry wt was determined for isolates 70-2, 50-2, R3-A, R1-P, and R2-P (Fig. 2). The three *A. klebsiana* isolates have similar optimum growth temp (27 and 30 C) as indicated by both dry wt and linear growth tests. The dry-wt basis and linear-growth basis differed slightly as a measure of optimum-growth temp for the *Pythium* isolates (R1-P and R2-P): maximum amount of mycelium was produced between 27-30 C in contrast to greatest linear growth at 30-33 C (Fig. 2).

Tests were carried out in controlled-temp tanks to determine whether the pathogenicity of *Achlya* isolates and *Pythium* isolates was affected by different temp. These experiments also allowed observations on seedling vigor at different temp in the absence of the organisms. Pathogenicity of the *A. klebsiana* isolates was less affected by different temperatures than that of the *Pythium* isolates (Table 4). In fact, the *Achlya* isolates were equally pathogenic throughout the temp range at which they grew well in culture. In contrast, the *Pythium* isolates grew well in culture at 30 C but were considerably less pathogenic at 30 C than at cooler temp (25, 20 C). The data for 15 C are not presented because seedlings did not grow at that temp (even after 25 days no seedlings had emerged from the control pots).

Effect of inoculation time on pathogenicity of A. klebsiana.—The conclusion that cooler temp adversely affect seedling vigor and delay emergence prompted us to conduct experiments to determine the effects of inoculation at increasing intervals after the seed were sown. These tests were carried out both in sterilized soil in controlled temp tanks at different temp and in unsterilized field soil placed in pots and watered to a depth of 2-3 inches. The seed used was soaked for different time intervals (12, 36, 60, and 84 hr) to provide germinating seed and young seedlings at different stages of development. Planting and inoculations were carried out on the same day for each of the groups of seed (i.e., various soaking times). Under these conditions, no significant differences in per cent emergence were noted. When seed were planted onto sterilized soil and inoculated at different time intervals, differences in per cent infection were again insignificant. Apparently the differences in seedling ages between 12 and 84 hr after initial soaking did not allow the seedlings to develop sufficiently to escape infection.

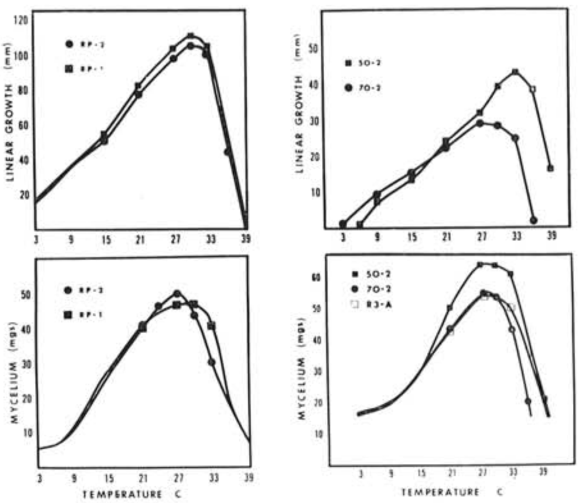

Fig. 2. Optimum temp for mycelial and linear growth of *Achlya* (50-2, 70-2, R3-A) and *Pythium* (RP-1, RP-2) isolates pathogenic to rice seed and seedlings.

Discussion.—The etiology and epidemiology of seed rot and seedling disease of rice in California is quite similar to that in other areas of the world. Various fungi are involved, but *Achlya klebsiana* and certain *Pythium* species are widespread and account for a major part of the disease syndrome.

Although none were encountered in the present study, it is possible that additional species of *Achlya* may be involved in the seedling disease of rice, since the culture conditions currently used in water-sown rice are ideal for the growth and development of these fungi. *Achlya klebsiana* appears to infect the seed coat and endosperm. Thus, failure of seed to germinate and failure of seedlings to emerge through the water may be due principally to a weakening of the seedling through utilization of the energy source (endosperm) by the fungus instead of direct damage to the germinating embryo. Further studies are needed to clarify this possibility.

Our results correlate with growers' observations that the seedling disease in California rice fields causes

Table 4. Effect of temp on pathogenicity of *Achlya* and *Pythium* isolates to Caloro rice seed and seedlings

	Tests[a] at each temp, C					
	20[b]		25		30	
Isolate	1	2	1	2	1	2
Achlya						
70-2	46.5[c]	66.6	46.8	45.7	52.2	31.7
50-2	67.5	72.3	48.9	62.1	47.7	46.3
Pythium						
R1-P	98.0	97.1	22.8	45.7	4.6	17.1
R2-P	98.0	89.2	56.6	69.6	23.1	24.3
Noninoculated	0	0	0	0	0	0

[a] Each test represents 4 replications/test, with 13 seeds/replication.
[b] At 20 C, the plants were consistently smaller and slower-growing.
[c] % Diseased plants based on differences in total healthy plants emerged in check and test pots after 23 days.

more damage when the temp at time of seeding is low. In our tests, rice did not germinate at 15 C, and plants grown at 20 C were always smaller and emerged more slowly through the water than when grown at higher temp. Although the *Pythium* isolates grew well in culture at 25 and 30 C, they produced very little seed and seedling damage in this range. It appears, therefore, that vigorously growing seedlings are resistant to or escape from attack by *Pythium*. *Achlya klebsiana* isolates, however, were about as pathogenic at high as at low temp. This difference in pathogenicity between *A. klebsiana* and *Pythium* may explain why only about 50% of seeds planted (current seeding rates of 50-70 seeds/0.092 cm^2 or 1 ft^2) produce plants even under warm conditions favorable for vigorous growth of seedlings.

Field trials of various fungicides as seed treatments applied prior to soaking of the seed have given marked increases in stands and have allowed a reduction in seeding rates. Captan and thiram at rates of 64 g/45 kg (2.3 oz/100 lb) seed are now recommended in California for control of seedling disease in water-sown rice.

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