STUDIES ON SAPROLEGNIACEOUS FILAMENTOUS FUNGI[1, 2]

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ABSTRACT

Seventeen species of saprolegniaceous filamentous fungi from Athens County were isolated, studied, and identified under pure culture conditions. Five of the 17 species have not previously been reported from Ohio (*Achlya caroliniana* Coker, *Saprolegnia kauffmaniana* Pieters, *S. mixta* de Barry, *S. hypogyna* Pringsh., and *S. monilifera* de Barry). Only five of the 17 species have previously been collected in southeastern Ohio. One hundred and twenty collections (15 a month for eight months—August through March 1968) yielded 113 isolates. Seventy-two percent were collected during August through October. *Saprolegnia ferax* was isolated most frequently. Most species, when grown on sterile hemp seeds in sterile glass-distilled water, initiated production of oogonia and antheridia sooner than those grown in a mixture of one third sterile lake water and two thirds glass-distilled water. Vegetative growth was greater in the latter medium.

INTRODUCTION

Crane and Vermillion (1966) published the only previous report on filamentous saprolegniaceous fungi (*Saprolegniaceae*) of southeastern Ohio. These workers collected eight species from the several ponds of the Southeastern Ohio Mental Health Center (formerly Athens State Hospital) adjacent to the Ohio University campus. Only five other reports on these fungi in Ohio have been published: Harvey (1952) recorded nine species from the little Miami River Basin in Hamilton, Clermont, Warren, and Clinton Counties; Cooke and Bartsch (1959, 1960) also collected nine species from the Little Miami River at Batavia and Williamsburg, from Lytle Creek in Clinton County, and from Mill Creek in Hamilton County; and Beneke and Schmitt (1961) and Schmitt and Beneke (1962) identified 24 species of filamentous saprolegniaceous fungi taken in the vicinity of South Bass Island in western Lake Erie. In the present study, 17 species from aquatic habitats in the environs of Athens, Ohio, were collected, purified, and identified.

METHODS

Monthly water collections (15 per month) were made for an eight-month period (August 1, 1967 through March 31, 1968). All collections were from aquatic habitats in Athens County. Most of the collections came from shore sites on Dow Lake in Strouds Run State Park; some collections were also made from Slater's quarry pond, from an unnamed pond on Johnson Road near U.S. Highway 33, and from "Lake View Pond" adjacent to the Ohio University campus. A total of 120 water samples were collected. Collection containers (40 ml capacity) were filled to capacity with the water sample and usually some associated organic debris and soil. Containers were returned to the laboratory, and all of the water and debris from each container was placed in separate standard-sized petri dishes (15 x 150 mm). Two halves of a sterilized hemp seed (*Cannabis sativa* L) were added to each petri dish, and the dishes were allowed to remain undisturbed for three days (at 21–26°C). The seeds were then examined microscopically for filamentous saprolegniaceous fungi. If these were found, pieces of hyphae were cut from the hemp seed and placed on F–13 medium, a very low-nutrient isolation medium (Difco agar, 20 gm; Difco peptone, 1.5 gm; Difco maltose, 0.04 gm and glass-distilled water, 1000 ml). From 12 to 24 hours later, hyphal tips which had grown away from contaminating bacteria (which usually remained concentrated near the area of inoculation) were cut off and placed on M–3 medium in petri

[1]Manuscript received August 28, 1968.

[2]Supported by National Science Foundation Grant GB 6855 to first author.

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plates (Difco corn meal agar, 17 gm; Difco peptone, 1 gm; Difco yeast extract, 1 gm; Difco glucose, 5 gm; Difco soluble starch, 5 gm; glass-distilled water, 1000 ml). In this way a pure culture was established for each fungus isolated.

One to two days later, some of the mycelium was transferred from the M–3 petri plate to an M–3 slant for a permanent stock culture, and a 6 mm disc of M–3 agar (cut with a sterile cork borer) with the fungus mycelium was placed in a sterile petri plate with just enough sterile glass-distilled water or (depending on the experiment) a mixture of one third filtered and sterilized lake water and two thirds sterilized glass-distilled water to cover the disc. One half of a sterile hemp seed was placed, cut end down, on the agar disc with mycelium. After the mycelium had penetrated into the hemp seed, generally two days later, the agar disc was removed from the hemp seed and from the petri plate.

Sometimes the stock slant and disc culture were prepared directly from the F–13 isolation medium, when the uncontaminated area of growth by the fungus on this medium was large enough to permit cutting the disc. The disc culture and later the hemp seed culture were incubated in a dark incubator at 20° C. After two days, sporangia were usually present and taxonomic data were taken which generally permitted generic determination. Species identification was made for each culture when the cultures produced oogonia and antheridia. Hemp-seed cultures for all isolates were discarded if they produced no oogonia or antheridia after sixty days.

TABLE 1

Saprolegniaceous filamentous fungi from southeastern Ohio

Species Collected	Collection Site*	Previously Collected in Ohio**
Achlya racemosa Hildebrand	DL	F
A. oblongata var. *oblongata* de Bary	DL, UP	A
A. prolifera C. G. Nees	UP	C
A. americana Humphrey	DL	A, C, D, E
A. klebsiana Pieters	DL, UP	C, D
A. flagellata Coker	DL	C, D, E
A. caroliniana Coker	UP	
Achlya sp.	DL, LV	
Saprolegnia diclina Humprhey	DL, UP, LV	D, F
S. kauffmaniana Pieters	DL, UP	
S. delica Coker	DL, UP, LV	A, E
S. ferax (Gruith.) Thuret	DL	A, C, E, F
S. mixta de Bary	DL	
S. hypogyna Pringsh.	DL	
S. monilifera de Bary	DL	
Saprolegnia sp.	DL, UP, SQ	
Aphanomyces laevis de Bary	DL	D, E
Aphanomyces sp.	DL, UP	
Leptolegnia caudata de Bary	DL	F
Dictyuchus monosporus Leitgeb	DL	A, C, D, F

*DL = Dow Lake
UP = unnamed pond
LV = Lake View Pond
SQ = Slater's Quarry

**A = Cooke and Bartsch (1959, 1960)
C = Beneke and Schmitt (1961)
D = Schmitt and Beneke (1962)
E = Harvey (1952)
F = Crane and Vermillion (1966)

RESULTS AND DISCUSSION

The 17 species collected during this study, together with their collection sites, are listed in Table 1. Included also in this table are the previous collection records of these fungi in Ohio. *Achlya caroliniana* and four species of *Saprolegnia*, *S. kauffmaniana*, *S. mixta*, *S. hypogyna*, and *S. monilifera*, have not previously

been reported in Ohio. Only five of the species identified during this study were previously reported from southeastern Ohio (Crane and Vermillion, 1966). These included *Achlya racemosa, Saprolegnia diclina, S. ferax, Leptolegnia caudata,* and *Dictyuchus monosporus.*

Twenty-nine isolates of *Saprolegnia,* eight isolates of *Achyla,* and two isolates of *Aphanomyces* failed to produce oogonia and antheridia and were therefore impossible to identify to the species level. All attempts to induce the production of the sexual reproductive structures in these sterile, hemp-seed cultures failed. Induction techniques used in attempts to influence production of oogonia and antheridia included: growth of the cultures in continuous darkness, in continuous light, or at naturally occurring laboratory photoperiods; growth of some cultures at 20°C and others at room temperature (24–27°C); growth in sterile glass-distilled water or in a mixture of one third sterile lake water and two thirds glass-distilled water; and growth with frequent changes (every two days) of water and with no changes of water. Usually most cultures in glass-distilled water initiated oogonial and antheridial growth within seven days, or failed to produce sexual structures regardless of how long they were maintained. Crosses in all possible combinations among the sterile isolates of *Achlya,* which genus has heterothallic strains, also failed to produce any sexual structures.

Five cultures of *Achlya klebsiana* required five weeks to produce oogonia and antheridia on hemp seed in a mixture of one third sterile lake water and two thirds glass-distilled water, while five similar hemp-seed cultures in glass-distilled water produced sexual structures in only five days. Generally speaking, hemp-seed cultures grown in glass-distilled water initiated production of oogonia and antheridia much sooner than did hemp-seed cultures grown in the mixture of lake water and glass-distilled water. Vegetative growth, however, was greater in the lake water-glass-distilled water mixture than in glass-distilled water alone. These results indicated that sterile glass-distilled water was satisfactory for growing most species of the Saprolegniaceae collected during this study, and that it induced the early production of sexual structures. These findings support the work of Dick (1965), who disagreed with Emerson's (1958) suggestion that plain distilled water generally provided a toxic environment for aquatic fungal growth. Dick found that glass-distilled water and autoclaved hemp seeds were not toxic for species of the Saprolegniaceae, Leptomitaceae, and Pythiaceae. Schmitt (1967) reported no appreciable growth differences in culturing some aquatic Phycomycetes regardless of whether undiluted lake water, a lake water-glass-distilled water mixture, or glass-distilled water alone was used. It is not clear from Schmitt's (1967) paper whether these results were obtained using pure cultures of the fungi studied. The present study also supported Emerson's (1958) report that vegetative growth was considerably enhanced in the lake water-glass-distilled water mixture. For identification purposes, however, vegetative growth in glass-distilled water was found to be adequate.

Table 2 includes information relating to the frequency of collection of these fungi during the eight-month period of this study and under the sampling methods described above. Certainly, such a short-range study can only give data of limited significance on the abundance and seasonal distribution of these fungi in the aquatic habitats studied. These data do, however, indicate something about the relative abundance of the species collected. For example, under this random method of collection of 120 water samples, *Saprolegnia ferax* was obtained twenty times; *Achyla flagellata* was collected only one time. Because *A. flagellata* was collected using the same methods as those for *S. ferax,* it may be concluded that, in the particular area, *S. ferax* was more abundant (produced more viable propagules—zoospores, gemmae, oospores). But the fact that *A. flagellata* was not collected after September also suggests that possibly it was unable to withstand the lower temperatures as well as *S. ferax.* On the other hand, there is the unlikely

possibility that the method of collection may have selected against the isolation of *A. flagellata* for some unknown reason.

Saprolegnia ferax was the most frequently collected water mold and was collected at least once in seven of the eight months. *Saprolegnia diclina* was the second most frequently collected species. Of the 113 isolates purified, 82, representing 72 percent, were collected during the three-month period, August through October, when the outdoor temperatures were relatively mild. Collections during the period, November through February, a time of freezing temperatures, when the collection sites were under ice and snow, totaled 28 species, representing only 20 percent of the total collections.

These collection data do not agree with the observations of Crane and Vermillion (1966) in their study of aquatic Phycomycetes from the ponds of the Athens State Hospital. They stated that "the species collected were most readily obtained

TABLE 2

Eight-month collection record of saprolegniaceous filamentous fungi from southeastern Ohio

Species Collected	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Total
Achlya racemosa	—	—	—	—	—	3	1	—	4
A. oblongata var. *oblongata*	2	1	—	—	—	—	—	—	3
A. prolifera	1	—	—	—	—	—	—	—	1
A. americana	—	—	—	—	1	—	—	—	1
A. klebsiana	1	—	1	—	—	1	—	—	3
A. flagellata	—	1	—	—	—	—	—	—	1
A. caroliniana	1	—	—	—	—	—	—	—	1
Achlya sp.	4	4	—	—	—	—	—	—	8
Saprolegnia diclina	7	4	1	—	1	1	—	—	14
S. kauffmaniana	1	1	3	—	—	—	—	—	5
S. delica	1	1	2	2	3	—	—	—	9
S. ferax	—	10	1	3	1	1	2	2	20
S. mixta	—	6	—	—	—	—	—	—	6
S. hypogyna	—	—	1	—	—	—	—	—	1
S. monilifera	—	—	—	—	1	—	—	—	1
Saprolegnia sp.	2	8	12	3	4	—	—	—	29
Aphanomyces laevis	—	1	—	—	—	—	—	—	1
Aphanomyces sp.	1	1	—	—	—	—	—	—	2
Leptolegnia caudata	—	2	—	—	—	—	—	—	2
Dictyuchus monosporus	—	—	—	—	—	—	—	1	1
Monthly Total	21	40	21	8	11	6	3	3	113

from November through April," and they reported a decrease in the number of aquatic fungi collected from May through September. They further suggested that this decline in the number of species collected during May through September was due to a seasonal decline. Data supporting this contention and methods of collection were not given.

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