AQUATIC FUNGI IN SOME OHIO STREAMS

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INTRODUCTION

The collection of aquatic fungi in Ohio has been confined to one published study (Harvey, 1952) and the material to be reported below as far as the literature of Ohio mycology is concerned. Because of ubiquity, we may assume that at least two species of these fungi occur in the state according to distribution records in the 1937 monograph of the Saprolegniales (Coker and Matthews, 1937). Studies in Michigan and Wisconsin have yielded lengthy lists of aquatic fungi but similar studies have not been of interest to Ohio workers. The following list of such fungi, found by the writers in an attempt to discover whether a relationship exists between polluted water and the aquatic fungi which may occur there (Cooke and Bartsch, 1959), is presented as a contribution to the lengthening check lists of fungi found in Ohio. Certain concurrent records from the Potomac River in Maryland related to the same question are included here. The streams and their various sampling stations are described fully in the cited paper by Cooke and Bartsch.

In addition to fungi found during the sampling efforts of the writers, those reported by Harvey are included in this listing. Several other records are included in order to bring the list up to date.

TECHNIQUES

The technique used by Harvey was relatively simple. A stream sample consisting of water and stream bed materials was placed in a culture dish in the laboratory and sterile cracked hemp seeds were added. Within several days, if aquatic fungi were present, their zoospores reached the hemp seed and there developed a colony in the form of a halo around the seed. This mycelium could be subcultured on fresh hemp seeds in sterile water in which carbon had been boiled and filtered off, or it could be subcultured on corn-meal agar slants. In the studies reported here, other techniques were used, involving the use of some kind of bait. Sterilized carbon water was found to be adequate for use as described above as far as contamination is concerned.

After a colony had developed on hemp seed for at least one, sometimes two weeks, it was in a robust state actively producing many sporangia and sometimes oogonia with oospores. In this condition the relatively fresh cultures of saprolegniaceous fungi frequently were transported to others for identification or checking of identifications. Either cotton or folded laboratory tissue was moistened, a hemp seed with its colony placed upon it, the tissue folded, the total wrapped in paper toweling and numbered. When preparing cultures for shipment, the primary wrapping material was damp enough so that it would not dry out in transit but not so wet that it would moisten the glue holding envelopes together. The group of cultures wrapped in paper toweling could be wrapped again in wax paper or aluminum foil and this package could be placed in a box, culture tube mailing can or envelope for mailing. Upon receipt of such material by cooperating specialists, the parcels were opened and planted again with fresh hemp seed. Usually, in the case of cultures wrapped in cotton, too many cotton fibers prevented immediate study while ones wrapped in tissue could be observed directly on being refloated in water.

At first, the baits used were enclosed in cylinders made of one-quarter inch

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mesh hardware cloth, stoppered at each end with corks. From one of the corks a steel wire skewer holding a piece of beef, a piece of apple, a whole date, or a rose hip or hawthorne fruit projected into the cylinder. The cylinder, floating partially submerged, was tied to some more or less permanent object on the shore and left for a week. Then the baits were transferred to a container filled with water from the sampling location, returned to the laboratory, and placed in sterile carbon water with sterile cracked hemp seed. Another technique followed a suggestion by Höhnk and Bock (1954). Bags of loosely woven nylon cloth, containing sterile, cracked hemp seed, were tied to the shore of the stream being sampled, and left for three days. These bags were returned to the laboratory in containers partly filled with water from the sampling site.

In one series of tests, aluminum tea balls were prepared in the laboratory by adding cracked hemp seed, assembling three on a string, wrapping, and sterilizing. These assemblies then were sent to a field survey team working on a tributary of the Potomac River. Each tea ball contained three hemp seeds and a multiple unit as described was used at each of six stations. One tea ball from each site was removed from the water after 2-, 4- and 6-day exposures. The tea balls, or their contents, were wrapped in absorbent tissue paper, then in absorbent toweling, the whole tagged and sent by regular mail to Cincinnati. In most cases, water molds were recovered from these collections by the use of fresh hemp seeds.

On the basis of this experiment, tea balls were used for the remainder of the test series. They were allowed to remain in the sampling station for 3–5 days depending on work schedules of persons involved in setting out and recovering samples. Experience showed that the shorter period of exposure was preferable.

Annotated List of Aquatic Fungi Isolated in Polluted Water Tests

Blastocladiales—Blastocladiaceae

Allomyces *spp.*—Isolated from soil samples obtained from clay bottom of basin dug near Lebanon, Ohio, as part of a stabilization pond sewage treatment system.

Isolated from contaminant of excess fish food in an aquarium at the Sanitary Engineering Center, Cincinnati.

Harvey reported isolating strains of this genus from Station 5.2 on Lytle Creek, Lytle Creek tributaries and from the East Fork of the Little Miami River and its tributaries.

Saprolegniales—Saprolegniaceae

Achlya *spp. nonsporulating.*—Isolated from stations 6 and 14 in the Potomac River, Maryland, following two days' exposure of hemp seed in tea balls and following 6 days' exposure. In Mill Creek, Ohio, it was found at station 4 in October, and in June it was isolated from stations 1, 2, 3 and 4. At one or more of these stations it was obtained on hemp seed, apple, human skin and insect larvae.

Achyla ambisexualis *J. R. Raper.*—Isolated from Mill Creek, Ohio, in October and June. In October it was found at station 1 on hemp seed, and in June it developed on hemp seeds at stations 1, 2 and 3.

Achlya americana *Humphrey.*—This was our commonest species of *Achlya* and was found on the Potomac River, Maryland, and in both Mill Creek and the Little Miami River in Ohio. On the Potomac River it was picked up at station 6 after 2 and 6 days' exposure of hemp seeds, at station 7 after 2 days, at station 14 after 2 and 4 days, at station 16 after 2 and 6 days, and at station 19 after 4 and 6 days' exposure of hemp seed in tea balls.

On the East Fork of the Little Miami River this species was isolated from water and on whole date bait on Nov. 18 and 25 below Batavia. At Williamsburg it was picked up in both sampling periods above town on apple, in the town on apple and date, and below the town on apple and date and it was isolated from water samples.

On Mill Creek this species was picked up in June at stations 1, 2 and 4 on hemp seeds, at stations 1 and 4 on apple, and at station 4 on human skin.

Harvey reported this species from the Lytle Creek basin.

Achlya bisexualis *Coker and Couch.*—Picked up in the Potomac River at station 6 after 2 days' exposure of hemp seed, and on Mill Creek in October at station 1.

Achlya dubia *Coker.*—On the East Fork of the Little Miami River this species was found above Williamsburg on apple, and below Williamsburg in a water sample. In Mill Creek it was collected at stations 1 and 2 on samples of human skin.

Achlya flagellata *Coker.*—Found at station 9 on the Potomac River after 2 days' exposure of hemp seed.

Harvey reported this species from station 8.7 on Lytle Creek, from tributary streams of Lytle Creek, from Stonelick Creek and from the east fork of the Little Miami River and its tributaries.

Achlya oblongata *de Bary.*—Found once on Mill Creek in October at station 4.

Achyla prolifera *C. G. Nees.*—On the Potomac River this species was isolated from station 16 after 2 days' exposure of hemp seed, and from station 19 after 4 days.

Aphanomyces laevis *de Bary* (?)—Found by Harvey at stations 4.2, 6.5, 7.6 and 8.7 on Lytle Creek, and in the Great Miami River near Ross (Venice).

Aphanomyces parasiticus *Coker on* Achlya americana *Humphrey.*—Found once at station 1 on Mill Creek in June.

Brevilegnia diclina *J. Harvey.*—Reported by Harvey from stations 5.2, 6.0, 7.6, 9.3 and 10.4 on Lytle Creek, from tributaries of Lytle Creek, Todd's Fork, Stonelick Creek and Reservoir, Cowan Creek, the Little Miami River, the East Fork of the Little Miami River and its tributaries, and the Great Miami River at New Baltimore and Miamitown.

Brevilegnia subclavata *Couch.*—Reported by Harvey from station 7.6 on Lytle Creek and from the East Fork of the Little Miami River.

Dictyuchus anomalus *Nagai.*—In the Potomac River at station 19 this fungus was found after 4 days' exposure of hemp seeds in tea balls.

Dictyuchus monosporus *Leitgeb.*—Isolated from the Potomac River on hemp seeds exposed at station 6 for 2 and 6 days, station 14 for 4 and 6 days, station 16 for 6 days, and station 19 for 2 and 6 days. At Lytle Creek, Ohio, this species was picked up on baits at station 7.2 in September, and at station 7.6 in January. On the East Fork of the Little Miami River this fungus was picked up on apple bait both above and below Batavia. At Williamsburg it was found at all sampling points. It was picked up on apple above town, on apple and date in town and below town on apple and date baits. On Mill Creek it was found in June at stations 1, 2 and 4 on hemp seeds exposed in tea balls.

Dictyuchus *sp.*—Harvey reported strains with and without oospores from stations 1.0, 5.2 and 8.7 on Lytle Creek, tributaries of Lytle Creek, Todd's Fork and the Little East Fork of Todd's Fork, Cowan Creek, the Little Miami River, and the Great Miami River at New Baltimore and Miamitown.

Geolegnia inflata *Coker and Harvey in J. Harvey.*—Reported by Harvey from the Lytle Creek Basin, Todd's Fork and Cowan Creek.

Geolegnia septisporangia *Coker and Harvey in J. Harvey.*—Reported by Harvey from station 7.6 on Lytle Creek.

Isoachlya *sp. nonfruiting.*—Obtained from station 19 on the Potomac River after 2 days' exposure of hemp seed.

Saprolegnia *sp. nonsporulating.*—Isolated from the Potomac River at station 14 after 2 days exposure of hemp seeds. In September on Lytle Creek this fungus was found on apple bait at stations 1.0, 2.8, 7.2 and 7.6; on dates at stations 1.0, 2.8, and 6.5; on meat at stations 1.0, 2.8, 4.2, 5.2 and 7.2; and on rose hips at stations 5.2, 6.5 and 7.2.

Saprolegnia delica *Coker.*—At Lytle Creek this fungus was found in September on rose hips at station 2.8, on dates at stations 4.2 and 7.6; and in January on several baits at stations 5.2, 7.6 and 8.7.

Harvey reported this species from stations 7.6 and 8.7 on Lytle Creek, Lytle Creek tributaries, Todd's Fork, Stonelick Lake, East Fork of the Little Miami River, a roadside spring near Lebanon and a small stream at Bridge No. 43, U. S. 22, near Montgomery, Ohio.

Saprolegnia diclina *Humphrey.*—Found in the Potomac River at station 6 after 6 days exposure of hemp seeds, and at station 14 after 2 days exposure.

Saprolegnia ferax (*Gruith.*) *Thuret.*—Collected on Lytle Creek in September on apple at stations 4.2, 5.2 and 6.5, at stations 4.2 and 8.7 on rose hips, on meat at stations 6.5, 7.6 and 8.7, and on dates at station 9.7; and in January at stations 1.0 and 7.6 on several baits. On the East Fork of the Little Miami River this species was found both above and below Batavia on apple, date and meat baits and in water samples. At Williamsburg this was found above, in and below the town on apple, and date baits and in water samples.

Harvey reported this species from stations 5.2, 6.5, 7.2 and 7.6 on Lytle Creek, Lytle Creek tributaries, Todd's Fork, and the West Fork of the East Fork of the Little Miami River.

Saprolegnia ferax-mixta *group.*—Several cultures isolated from hemp seed exposed in the Potomac River have been assigned here. These were obtained at station 6 after 2, 4 and 6 days' exposure, and at station 7 after 2 and 4 days' exposure. Two unidentified strains of *Saprolegnia* were obtained by Harvey in the Lytle Creek Basin and on tributaries of the Little Miami River.

Saprolegniales—Leptomitaceae

Leptomitus lacteus (*Roth.*) *Ag.*—Occasionally material assignable tentatively to this species has been found intermingled with other aquatic fungi. It has not yet been obtained in pure culture. Lytle Creek in September on rose hips at station 2.8, on dates at station 4.2 and on apple at station 7.6, and in January at stations 2.8 and 8.7 on various baits. On the East Fork of the Little Miami River this was found at Batavia above town on apples and dates, and at Williamsburg in town on apple bait.

Lagenidiales—Olpidiopsidiaceae

Olpidiopsis fusiformis *Cornu on* Achlya americana *Humphrey.*—This fungus is an internal parasite in the mycelium of its host. It was found once on Mill Creek at station 2 in June.

Olpidiopsis saprolegniae (*Braun*) *Cornu on* Saprolegnia ferax.—This parasite was found at Potomac River station 6 after 4 days exposure of hemp seed.

Olpidiopsis saprolegniae *var.* levis *Coker on* Saprolegnia ferax.—Found once on hyphae of the host in the East Fork of the Little Miami River in Williamsburg on date baits.

An olpidiaceous parasite on *Dictyuchus monosporus* was found after exposing hemp seed at station 19 in the Potomac River for 6 days. Material was inadequate for further study.

Peronosporales—Pythiaceae

Pythium debaryanum *Hesse.*—Harvey reported isolating this species from Lytle Creek tributaries, Cowan Creek, the East Fork of the Little Miami River, and from the Great Miami River at New Baltimore.

Pythium torulosum *Coker and Patterson.*—Harvey reported isolating this species from Cowan Creek and from the East Fork of the Little Miami River.

Pythium ultimum *Trow.*—Harvey reported isolating this species from Cowan Creek and from the East Fork of the Little Miami River.

Pythium *sp.*—Found in the Potomac River at station 6 after 2 and 6 days exposure of hemp seed, at station 9 after 2 days exposure, and at station 19 after 4 days exposure. On many attempts to recover water molds from Lytle Creek samples, using hemp seeds in bottom deposits brought to the laboratory, several strains of this genus have been found but were not traced to species because most species in the genus are identified partly on the basis of association with a host plant in the soil.

Harvey reported isolating unidentifiable cultures of *Pythium* from the same streams reported under *P. torulosum.*

Peronosporales—Zoopagaceae

Zoopagus insidians *Sommerstorff.*—Appearing occasionally in activated sludge systems at Sanitary Engineering Center as reported recently (Cooke and Ludzack, 1958).

SUMMARY AND CONCLUSIONS

In view of the number of aquatic fungi reported from North America in the literature of the water mold specialist, and in view of the wide diversity of stream

conditions and aquatic habitats in Ohio, it is suggested that much information could be obtained on the populations of these organisms within the state. Streams flowing from glaciated to unglaciated areas, crossing new valleys through the older glaciated areas, and flowing through the hills in unglaciated areas traverse wide varieties of geological formations each of which affects the stream in one way or another. Farm ponds, fishing lakes, recreation lakes, and natural lakes as well as roadside ponds, ditches and streams, could furnish the collector or observer with a wide variety of habitats. Aquatic fungi also occur in soils and this field is completely untouched in Ohio. From upland beech forests and fields resulting from their clearing, to Cedar swamps, undisturbed hemlock forests, limestone canyons and sandstone conglomerates, to coal mines and the acid seepage and drainage waters issuing from them as well as in intermediate areas of many kinds, there are many habitats which deserve careful study.

As a corollary to the present study an attempt was made to obtain chitrids by the use of pollen affixed to slides in petroleum jelly. Probably because of the short exposure period no success was experienced. However, this technique could be tried in a number of habitats using a number of variables to determine something of the population of such organisms in the state.

It is suggested that persons making surveys of any type of body of water for aquatic fungi of any kind record water quality data as part of the survey to show something of the habitat and to aid in pollution studies. Additional suggestions include varying the length of exposure time in the field, method of sampling, types of bait, types of bait holders, comparisons of the validity of bringing habitat to the laboratory and taking bait to the habitat, populations at different depths in a lake in relation to the water zones within the lake, and any other factor which appears essential to a fuller understanding of our knowledge of aquatic fungi in their environment.

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