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AQUATIC PHYCOMYCETES OF RADNOR LAKE, NASHVILLE, TENNESSEE

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ABSTRACT

Collections of aquatic phycomycetes were made at Radnor Lake, Nashville, Tennessee, an area chosen because of current local interest. Collections made from July, 1973, through December, 1975, resulted in identification of 13 species of water molds. Those found were *Olpidiopsis saprolegniae* var. *saprolegniae, Aphanomyces laevis, Aphanomyces spp., Dictyuchus monosporus, Achlya americana, Achlya proliferoides, Achlya rodrigueziana, Achlya spp., Saprolegnia ferax, Saprolegnia diclina, Saprolegnia anisospora, Saprolegnia subterranea, Saprolegnia litoralis, Saprolegnia spp., Pythium proliferum, Pythium irregulare,* and *Pythium spp.* Several of these have not been previously collected in Tennessee.

Information was accumulated on the seasonal periodicity of species. Species with centric or subcentric oospores were characteristic of cooler months, while those characterized by eccentric oospores were present all year, but predominated in summer. These results compare well with the findings of other workers.

INTRODUCTION

Although a wealth of information has accumulated concerning occurrence of aquatic phycomycetes in many areas, reports of them in Tennessee are scarce. Raper (1936) collected *Achlya bisexualis* Coker and Couch in Tennessee. Shanor (1937) used an isolate of *Thraustotheca clavata* (de Bary) Humphrey collected in Tennessee in 1935 in his cytological study of the reproductive structures. Hutchison (1940) collected and identified 13 species in the vicinity of Nashville.

Whereas the usual procedure in collecting these organisms involves sampling from a variety of sites, this report is restricted to Radnor Lake, Nashville, Tennessee. Much local interest in Radnor Lake has been generated in recent years because of its acquisition by the State of Tennessee as a preserved Natural Area. It is an 80 acre lake within the city limits of metropolitan Nashville (McCall, 1975).

As the collections in this study were made throughout the calendar year, it seemed of interest to relate the periodicity of species in this collection and those of Hutchison (1940) to those found by other workers. Hughes (1962) felt that the Saprolegniaceae of the southeastern United States could be divided into two seasonal groups. Species producing centric and subcentric oospores, regardless of generic affiliation, showed a definite seasonal periodicity, generally occurring more commonly in cooler months. Species producing eccentric oospores showed no apparent seasonal pattern. Hughes also presented data indicating that eccentric oospores characterize tropical species, while in temperate regions species with centric or subcentric oospores make up an increasingly larger percentage of reported collections. Clausz (1974) attempted to shed light on possible causes of seasonal periodicity of Saprolegniaceae in Muskrat Pond of the North Carolina Botanical Garden, Chapel Hill, North Carolina, and has presented an excellent bibliography of seasonal periodicity studies of water molds.

MATERIALS AND METHODS

Collection and isolation techniques employed were similar to those given by Johnson (1956) and by Seymour (1970). Water samples were collected at approximately monthly intervals in 100 ml screw cap vials. Collections were made initially both at the water's edge and at varying depths at considerable distance from shore, but as the latter yielded negligible results, subsequent collections were made only in water a few inches deep or from damp soil near the water's edge. Samples were taken both from the lake proper as well as from the three stagnant sloughs which do not connect to the lake itself (McCall, 1975). Attempts were also made to collect fungi by use of screen wire traps baited with bits of apple, dead insects, twigs, corn grains, and other items, and by submerging sponges and pieces of plexiglass into the lake. However, most isolates reported here resulted from water collections baited in the laboratory.

Most water samples were plated in the laboratory by pouring approximately 30 ml of sample into sterile petri plates and baiting with four or five boiled hemp seed halves. Other baits used with some success were snakeskin and roach wings. Pine pollen and cellophane were also used but yielded no successful isolations.

Aquatic phycomycetes appeared in 145 of 207 collections made. Since many of these were mixtures of more than one species it was necessary to isolate each fungus into axenic culture before it could be identified to species. To facilitate this, a single hypha of 1-2 mm was cut from the colony with the aid of a stereomicroscope, washed through several changes of distilled water, and placed on sterile corn meal agar plates. Occasionally a sporangium, zoospore, or oogonium was transferred instead. Usually in 2-3 days mycelia appeared and in most instances outgrew any bacteria present. An agar block of about one square centimeter containing only hyphal tips of this mycelium was then placed in another plate of corn meal agar, resulting in an axenic, unifungal culture.

Production of reproductive structures was achieved by removing a cube of overgrown agar, placing it in a petri plate of distilled water, and introducing a boiled hemp seed half on top of the mycelium on the cube. After 2-4 days mycelial growth extended from the seed and a unifungal culture was maintained.

Coker (1923), Matthews (1931), Coker and Matthews (1937), Middleton (1943), Johnson (1956), Sparrow (1960), and Seymour (1970) were the authors of monographic works used in identification.

FUNGI COLLECTED

Lagenidiales

Olpidiopsis saprolegniae var. *saprolegniae* (Braun) Cornu. This holocarpic endoparasite was observed eight times. Sporangial discharge was observed, as were resting spores with spiny walls, sometimes with smaller, clear companion (male?) cells attached.

This variety differs from *Olpidiopsis saprolegniae* var. *levis* in that the former produces resting spores with spiny walls, while the latter produces only smooth-walled resting spores.

Repeated attempts were made to culture several of the *Olpidiopsis* isolates by placing infected hyphae with other hemp seed cultures of *Saprolegnia*, hemp seed cultures of several species of *Achlya*, and cultures of these on corn meal agar. Only the first met with any success.

Saprolegniales

Aphanomyces laevis de Bary. This species was isolated four times on snakeskin and hemp seed. Coiling of antheridia around hyphae subtending the oogonial stalk or the oogonial stalk itself was practically universal.

Dictyuchus monosporus Leitgeb. This species was isolated twelve times from twigs and hemp seed. All isolates produced true net sporangia from which zoospores escaped singly through the numerous openings in the sporangial wall (Figs. 1, 2, 3).

FIG. 2. Dictyuchus monosporus *Leitgeb. Same sporangium as Fig. 1 a few seconds later, three zoospores having been discharged. x400*

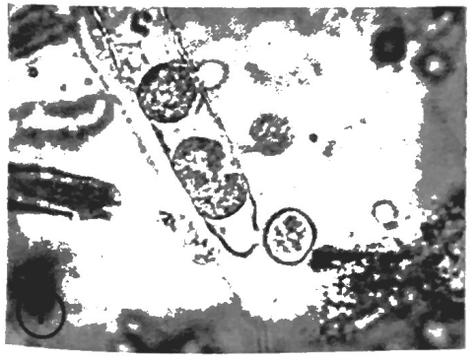

FIG. 1: Dictyuchus monosporous *Leitgeb. Net sporangium showing one zoospore that has just escaped, and two others in the process of discharge. x400*

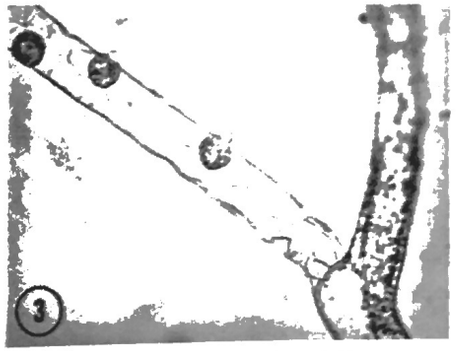

FIG. 3. Dictyuchus monosporus *Leitgeb. True net sporangium. Note opening in sporangial wall through which zoospores have already been discharged. x250*

Achlya americana Humphrey. This species was isolated seven times from Radnor Lake.

Achlya proliferoides Coker. This species was readily identified by diclinous antheridia that coiled around the hyphae for some distance in approaching the oogonium, but not around the oogonial stalks themselves. One isolate which fit these criteria was obtained on hemp seed from twigs incubated in a wire trap. Upon rebaiting, however, sexual structures were not produced. Instead, a culture was obtained containing dark, spherical gemmae. These were extremely abundant, and often intercalary and catenulate. At least two other isolates were perhaps also *Achlya proliferoides*, as the same sterile structures were abundantly produced.

Achlya rodrigueziana Wolf. One isolate displayed many of the features of *Achlya rodrigueziana*, differing only in oogonial wall characteristics. Possession of diclinous antheridia coupled with a small number of eccentric oospores per oogonium (usually 1-2) distinguishes this species from those closely related. The oogonia had unpitted walls, frequently exhibited small, rounded projections of the oogonial wall surface, and frequently proliferated from older oogonia (Fig. 4). Empty oogonia appeared to be irregular on their inner surfaces.

FIG. 4: Achlya rodrigueziana *Wolf. Proliferated oogonia with papillae. x250*

The only other *Achlya* species characterized by eccentric (or nearly so) oospores is *Achlya orion* Coker and Couch, which has androgynous antheridial branches. Except for the papillae on the outer surface of the oogonia, this isolate fits reasonably well the published descriptions of *Achlya rodrigueziana*.

Saprolegnia ferax (Gruithwisen) Thuret. This common and widely distributed species was isolated four times on hemp seed. Isolates often produced oospheres within old sporangia or hyphae (Fig. 5).

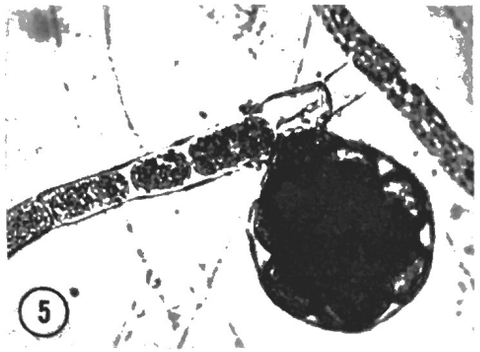

FIG. 5: Saprolegnia ferax *(Gruithwisen) Thuret. Oogonium and supporting hypha with oospheres present in supporting hypha as well as oogonium. x250*

Saprolegnia diclina Humphrey. *Saprolegnia diclina* was isolated six times from Radnor Lake.

Saprolegnia anisospora de Bary. This species was readily segregated from other species of the genus found at Radnor Lake by its eccentric oospores.

Saprolegnia subterranea (Dissman) Seymour. This species usually produced one oospore per oogonium (occasionally two) and was thus easily distinguished from other species of the genus. A small percentage of the oogonia regularly produced one or several papillate projections of the oogonial wall (Fig. 6).

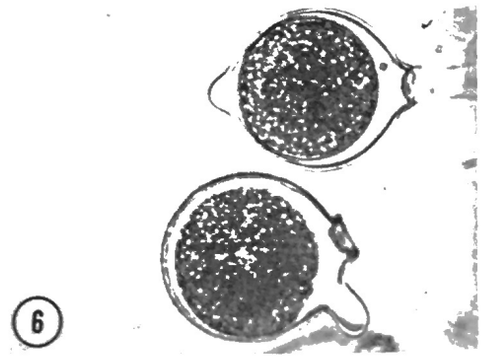

FIG. 6: Saprolegnia subterranea *(Dissmann) Seymour. Oogonia showing apiculate oogonial wall. x250*

Saprolegnia litoralis Coker. One isolate was obtained on hemp seed.

Peronosporales

Pythium proliferum de Bary. This species was characterized by spherical sporangia which renew themselves by internal proliferation, and by oospores which are aplerotic. It was isolated on hemp seed and easily maintained on corn meal agar at 4°C for over twelve months.

Pythium irregulare Buisman. Characterized by a spherical sporangium and an irregular oogonial wall, this species was isolated once on hemp seed.

Many isolates produced sporangia which allowed identification to genus, but did not produce the sexual reproductive structures necessary for identification to species, even after several months. Thus 58 isolates were categorized as *Aphanomyces sp., Achlya sp., Saprolegnia sp.*, or *Pythium sp.* Those isolates which produced oospores were included in the data on periodicity of occurrence even though they could not be identified to species.

Periodicity of Occurrence

Of the samples collected, 61 isolates were obtained which eventually produced oospores. Seasonal periodicity related to oospore type seemed to correlate generally with the findings of Hughes (1962). Species characterized by centric (or subcentric) oospores (mainly species of *Saprolegnia*) were somewhat more predominant in cooler months, while eccentric species were present at all seasons of the year (*Achlya americana, Dictyuchus monosporus*) but especially prevalent in summer months (*Aphanomyces laevis, Dictyuchus monosporus*). Figure 7 illustrates the seasonality of species with regard to oospore type. Although collections were generally made more often than monthly, inadvertently none fell in the calendar months of April and August.

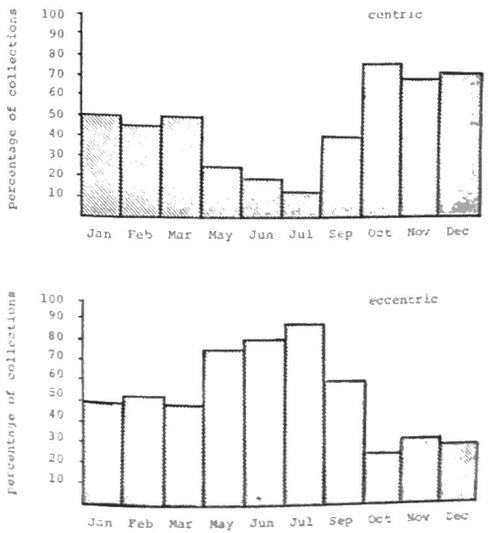

FIG. 7: *Percentage frequency by oospore type. No data for April and August. Includes data at Radnor Lake and of Hutchison* (1940).

Discussion

Previous workers have shown that aquatic phycomycetes may be isolated from practically any lake, stream, or soil sample, with hemp seed or other suitable bait. Radnor Lake does not present an exception in this regard.

The pH of Radnor Lake was 7.6 or above at the time of several collections. Of the species found, several have often been shown to inhabit alkaline or neutral waters. Lund (1934) in Denmark listed *Dictyuchus monosporus, Saprolegnia ferax, Achlya americana*, and *Aphanomyces laevis* in water of pH 6.5-8.4. Wolf and Wolf (1941) in Florida found *Achlya americana, Achlya proliferoides, Aphanomyces laevis, Dictyuchus monosporus, Saprolegnia delica* (now *Saprolegnia diclina*, Seymour, 1970), *Saprolegnia ferax*, and *Olpidiopsis saprolegniae* at pH 7.2-7.6. Roberts (1963) in Great Britain found *Saprolegnia ferax* and *Aphanomyces laevis* above pH 7.8, but found *Achlya americana* in a pH range of 3.6-6.4. Thus by far the majority of species found at Radnor Lake, and all of those often collected, have previously been found to predominate in waters of alkaline or neutral reaction. It seems likely that a narrow pH range limits diversity, but it probably is not a decisive factor, as some species such as *Achlya americana* seem to have a wide range of tolerance to hydrogen ion concentration.

In addition to seasonality of oospore type, however, several other findings concurred with those in the literature. *Saprolegnia diclina* and *Saprolegnia ferax* were most common from September through December and not found during the coldest months, January and February. Clausz (1970) observed this to be the case with *Saprolegnia diclina*. He also noted *Achlya americana* to be absent in summer months, while *Aphanomyces laevis* and *Aphanomyces spp.* were very abundant in the summer. His observations coincide with the present findings at Radnor Lake, with respect to absence of *Aphanomyces* in all but the summer months and the presence of small numbers of *Dictyuchus* throughout most months of the year.

It is probably of more interest than significance that the only isolate of *Saprolegnia* producing eccentric oospores, *Saprolegnia anisospora*, was found in the summer. All eight isolates of *Olpidiopsis* were found in cooler months, October through February. This may be significant only in that the *Olpidiopsis* isolates were found on *Saprolegnia*, which predominated in cooler months.