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AN INVESTIGATION OF AQUATIC OOMYCETES IN FIVE AREAS OF PLEASANT PRAIRIE TOWNSHIP

by

Frederick L. Schmude

ABSTRACT

The purpose of this study was to determine the types of Oomycetes present in selected aquatic habitats in Pleasant Prairie Township, Kenosha County, Wisconsin. Pleasant Prairie was selected because no previous studies of this nature had been made there.

The five habitats chosen were the Des Plaines River, Lake Michigan, Barnes Creek, Mahoney' Creek and Mahoney's Pond.

The method of obtaining the samples were a modification of the trapping techniques proposed by Patterson in 1967 and Nelson in 1969. Baby food jars containing either hemp seeds or dead flies were placed in a styrofoam float and covered with cheese cloth. They were then partially filled with water and inverted mouth down in the surface water of the selected site.

Through the use of the traps and pure culture techniques on

Difco Corn Meal agar, it was determined that there were at least five types of Oomycetes present. The organisms found were *Saprolegnia ferax* (Gruithuison, Thuret), *Saprolegnia diclina* (Humphrey), *Apodachyella completa* (Humphey, Indoh), *Dictyuchus* sp., and *Pythium* sp.

Selected micrographs have been included to illustrate the structures involved in classifying these organisms.

AN INVESTIGATION OF AQUATIC OOMYCETES IN FIVE AREAS OF PLEASANT PRAIRIE TOWNSHIP

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by

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INTRODUCTION

Pleasant Prairie Township is one of eight townships located in Kenosha County. It is bounded on the East by Lake Michigan; North by 60th Street; West by Highway I-94; and South by the Illinois-Wisconsin state line. The Township covers an area of approximately fifty-six square miles of low, flat land primarily used for farming.

Located within this area are several small spring-fed creeks, the Des Plaines River and one spring-fed pond. This investigation will include five sampling areas. Three of the five sampling areas are basically surrounded by farm land, the other two by unused land with some residential units nearby.

The first sample area was on the North side of Mahoney's Creek; the second was on the North side of Mahoney's Pond which drains into Mahoney's Creek; the third was on the West side of the Des Plaines River; the fourth along the South side of Barnes Creek; and the fifth was on the East shore of Lake Michigan.

The purpose of this study is to investigate the types of aquatic Oomycetes common to the surface layer of each sampling site in these areas by bi-weekly intervals during the months of August, September, October, November, April, May and June.

LITERATURE REVIEW

According to Wolf and Wolf (1947), if fungi are among the "simplest of organisms" their classification should not be too difficult. The student, however, is forced to conclude that these organisms are far from simple. If simplicity was the key characteristic the numerous volumes written need not exist.

All classifications are man-made and therefore contain flaws. The ideal classification has not been, nor shall be, proposed until researchers reveal morphological similarities and differences between species and indicate their evolutionary position and geneological relationships (Wolf and Wolf, 1947).

During an address to the Mycological Society of America, G. W. Martin (1955) proposed this question, "Are fungi plants?" After an extensive review of the literature he could only advance reasons for differences of opinion and promote the idea that though differences exist the roadblocks which hamper the progress to the phylogeny of the fungi be removed.

The classification of organisms into the two kingdoms, Plantae and Annimalia using the binomial system stems from the work of Linneaus to Haeckel, who proposed in 1866 a third kingdom Protista which was to include all organisms not clearly plant or animal. This has been expanded by Copeland in 1956 to a fourth grouping, the Monera (Alexopoulos, 1962) which is advocated by many modern authors in their general biology texts (Weisz, 1967; Curtis, 1968; and the BSCS staff 1960, 1968).

The fungi, irregardless of the major taxa to which they are placed are classified in what appears to be two major schemes depending upon the source used. Alexopoulos (1962) classified the fungi into Division Mycota, with two sub-divisions; the Myxomycotina and the Eumycotina. In his scheme he gave the group of organisms under study, the Oomycetes, the rank of class. However, Wolf and Wolf (1947), Bessey (1935), Fitzpatrick (1930), Langeron and Vanbreuseghem (1965) and Sparrow (1960) rank the Oomycetes as a subclass in the class Phycomycetes.

The Oomyceteae are divided into two to five orders depending upon the source used. All authors consulted recognize the Saprolegniales and Peronsporales with Fitzpatrick (1930), Bessey (1935), Wolf and Wolf (1947) and Langeron and Vanbreuseghem (1965) including the Monoblepharidales, whereas, Alexopoulos (1962) includes this group in the class Chytridiomycetes. Other orders that have been variously included in the Oomyceteae are: Leptomitales (Wolf and Wolf, 1947; Sparrow, 1960; Alexopoulos, 1962; and Langeron and Vanbreuseghem, 1965; Pythiales, (Wolf and Wolf, 1947); and Lagenidiales, (Sparrow, 1960; and Alexopoulos, 1962).

Basically the Oomyceteae consist of those fungi that reproduce asexually by biflagellate zoospores that are borne in sporangia of various types, except in the most advanced species in which the sporangium assumes the function of a spore and germinates directly by a germ tube (Sparrow, 1958, 1960; Alexopoulos, 1962; Langeron and Vanbreuseghem, 1965).

Sexual reproduction ranges from the entire gametangium being formed from the whole thallus to heterogamy. The formation of oospores is characteristic of all but the most primitive species. The oospores originate and mature within the oogonia (Sparrow, 1960; Alexopoulos, 1962).

Structurally, this class consists of organisms that range from a primitive unicellular thallus to a profusely branched, filamentous mycelium. The majority of the Oomycetes are eucarpic with the more primitive being holocarpic. These organisms are generally saprophytic, though there are many that are parasitic with the highest forms being terrestrial obligate parasites (Sparrow, 1958, 1960).

There are two generally accepted methods of identifying the Phycomycetes: the older method used by Fitzpatrick (1930) consists of examining the mycelium, the thallus, and then the sporangium and gametangium; the newer method, which is a distinct departure, estab- by Sparrow (1958, 1960) involves examining the flagellation of the zoospores, then the thallus and finally the gametangium.

There are several examples of a "typical" life cycle for the Phycomycetes in general and the Oomycetes in particular. Raper's (1939-1951) classical investigation of the sexual mechanism of Achlya bisexualis Coker and Achlya ambisexualis Raper is heavily referred to, however, the dioecious condition is not prevalent enough in this class to consider it a representative of this class (Figure 1).

In the somatic portion of Saprolegnia, the thallus is composed of two types of hyphae; the rhizoidial type found in the substratum; and the branced hyphae that form the visible colony, where the reproductive organs are found. Depending upon environmental conditions, either the sporangium or the gametangium will appear (Wolf and Wolf, 1947; Elliott, 1968; Moore, 1968). The zoospores demonstrate diplanetism while the oogonia, which contain one to many oospheres, will form thick walled diploid oospores (Couch, 1941; Sparrow, 1958, 1960; Alexopoulos, 1962).

Ecologically, few specific studies have been done. Information is usually gained from the accounts of the morphology, taxonomy and physiology of specific fungi. The area of pollution and environmental studies has given new impetus to the study of fungi in water systems and should add ecological information concerning the Phycomycetes.

The qualitative composition of fungal populations, the substrata, and their place and role in the aquatic environment is fairly well known. Which organisms and their precise functions in degradation of organic materials is less well known (Sparrow, 1968).

Quantitatively there is very little known about the Phycomycetes. How significant and effective they are in decompostion; how many organisms exist per given volume; their distribution, both horizontally and vertically as to stratification in water; their relationship and number in the benthos is not known (Sparrow, 1968).

The aquatic Phycomycetes can be found in pools, lakes, ponds, rivers, streams, estuaries and other marginal areas in some form. To date no fungi have been isolated from thermal springs, though sphagnum bogs with their low pH support a rich and diverse variety of Phycomycetes such as Achlya, Pythium and Phlyctochytrium (Miller, 1965). Thraustochytrium has been found in salt lakes (Sparrow, 1968) and Saprolegnia and Achlya in acidotrophic lakes (Suzuki, 1960).

Factors found in the habitat that affect these organisms to some degree are: turbidity; light; substratum; temperature; oxygen, pH; altitude and seasonal changes.

The amount of solid material in suspension can affect the distribution of the organisms and substrate availability. Light affects these organisms indirectly through temperature, algal growth on substrata, and in some cases, the reproductive cycle, i.e., Blastocladiella emersonii (Cantino and Hyatt) and Blastocladiella britanica Horenstein and Cantino, (Horenstein and Cantino, 1961).

Substratum availability and character are important to heterotrophs. It has been found that many Phycomycetes are selective in the laboratory (Whiffen, 1941). It has been found that the zoospores have a chemotactic ability and that they can be affected by age and solutes available (Sparrow, 1968).

Temperature directly affects germination, vegetative growth and reproduction. Temperature ranges vary between organisms, but in general, fungal structures have been found functioning between six degrees Centigrade in Blastocladia (Cotner, 1930) to twenty-five degrees Centigrade in Achlya (Reischer, 1949). The relationship of temperature to the induction of morphological structures of taxonomic importance has been shown to be a promising new area of investigation (Sparrow, 1968).

Aquatic Phycomycetes vary greatly in the amount of oxygen needed for nutrition and growth. It has been shown that certain species of Apodachlya and Sapromyces, require an oxygen-rich environment and some species of Blastocladia and Rhipidium will grow where there is little oxygen present and the environment is foul, depending upon the particular metabolism of the organism (Emerson and Cantino, 1948; Sparrow, 1968).

Aquatic fungi have been found in habitats ranging in pH from 1.9 to 8.0, through the general range is a pH of 5.6 - 7.4. Aquatic pH cannot be considered by itself but must also be considered in relation to the dissolved carbon dioxide and bicarbonate which play an important role in pH change (Roberts, 1963).

It has been demonstrated that the aquatic fungi follow a seasonal occurrence with spring as the most favorable season for growth. During the summer, when the temperature increases, water levels fall and pH decreases, the Phycomycetes show the greatest decline. In the autumn, with the advent of lower temperature, more water and an abundance of substratum, there is a renewal of growth (Sparrow, 1968).

There is little evidence of ecological succession though it has been commonly assumed to occur. Also, it is not known whether species that occur at the same time form communities or are just aggregations of individuals (Sparrow, 1968). Much work and investigation needs to be done in this area in order to fully understand the life patterns of the aquatic Phycomycetes.

The class Oomycetes which includes the water mold, white rusts and downy mildews have rather diverse habitats (Alexopoulos, 1962). The Lagenidiales comprise a group of primarily microscopic, endobiotic parasitic fungi that are found in fresh and marine water. Species of Olpidiopsis and Rozellopsis for example, are parasitic on aquatic Phycomycetes and several algal forms. Very little work has been done to ascertain the complete range of hosts which these fungi will attack.(Sparrow, 1960).

The Leptomitales are the only group that have been found solely in fresh water as saprophytes occurring on vegetable debris. They are found in both cool, clear water and under exceedingly polluted conditions (Sparrow, 1960; Willoughby and Collins, 1966). They differ from the other Oomycetes in that they are regularly septate, and except in *Apodachlyella completa*, contain only one oosphere (Sparrow, 1960).

The Peronosporales comprise a primarily terrestrial, microscopic fungi parasitic for the most part on flowering plants. The Pythiaceae is the only family that displays a range of habitats from aquatic to amphibious and terrestrial. It is felt that the majority of these organisms are able to lead a saprophytic existence. They are generally identified by the characteristics of the sporangia and the sporangiophores. *Pythium* sp. is an example of the sporangial type and *Peronospora* sp. would be an example of the advanced sporangiophorial type (Sparrow, 1960; Alexopoulos, 1962).

The term water mold is customarily used to designate the Saprolegniales. The majority of this group are aquatic saprophytes, though many species are soil-inhabiting and some are parasitic on both plants and animals. Structurally, they range from one-celled, holocarpic organisms parasitic on algae to those with well-developed coenocytic, non-septate mycelium,, except for differentiation of the reproductive structures. These organisms have been found on a world-wide basis.

Because of their distribution, ease of growth and adaptability among other items, the Phycomycetes have played a role in studies of genetic, cytology, taxonomy and morphology. The oomycetes have been studied to some extent in these various areas. Current literature covers topics such as genetics, where it has been found that meiosis does not occur in the germinating oospore (Ziegler, 1953) but rather in the gametangia (Mullins, 1968). Mullins (1968) also found that water molds other than Achlya sp. had reproduction initiated and controlled by hormones. Clausz (1968), besides describing a different method of classification based on oospore structure, felt that temperature and light also played an important part on oogonial structure and reproduction. Most of the work done has stemmed from the work done by Raper (1940, 1947, 1951) and Barksdale (1960,1962,1963, 1966).

Gleason (1968) has done excellent work on the nutritional aspects of the Leptomitales. He has found that in this group there was no single carbon source that was adequate for growth of the organisms tested, however, asparagine was found to take care of the nitrogen requirements of all tested species.

Taxonomically, much work has been done on this class. Couch (1938, 1941) describes techniques of flagellar staining and descriptions of zoospores that had a bearing on Sparrow's (1958, 1960) work. Hendrix (1968) has described a new species of Pythium based on heterothallism and Karling (1968) has added the genus Aphanomycopsis in the Saprolegniaceae near Aphanomyces as two examples of work recently done taxonomically.

As a group the aquatic Oomycetes have not been shown to be ecologically or economically important. Several exceptions to this would be the fish and crayfish molds (Unestam, 1968; Stuart and Fuller, 1968) and some of the amphibious forms of the Pythiales (Alexopoulos, 1962). It is felt that the distribution of the Saprolegniales is of wider occurrence than just freshwater. Dick (1968); Stuart and Fuller (1968) using both literature review and experimentation believe that this group is quite prevalent in mixed saline waters of estuaries.

Studies done on the effect of chemical and mechanical agents in an attempt to understand the organism to control it are quite enlightening. Hickman and Ho (1966) found that zoospore attraction of the Pythiales can be altered by application of chemicals, urea, growth substances and antibiotics. Unestam (1968) found that the enzyme chitinase of Aphanomyces astaci Schikora was affected by the metalic ions of absorbtion of chitinase by chitin protected against shaking, heating and pH changes.

Martin (1968) in his esperimental work showed that malachite green was effective against various Saprolegniales in dosages ranging from 5 ppm/5 minutes to 15 ppm/hour depending upon the species, whereas, acriflavine only started to become effective at 100 ppm/hour.

Gysan (1969) reports that a chelated organic copper compound (cutrine) developed by Dr. B. Domogalla effectively controls Saprolegnia Sp. with no fish loss when applied at $\frac{1}{4}$ ppm in a drip system. Barrick (1969) reported that copper sulfate used as a fungicide was deadly to trout at a concentration as low as 0.14 ppm whereas copper in the form of an organic compound killed fish when applied in excess of $\frac{1}{2}$ ppm copper ion yet was effective in controlling Saprolegnia at $\frac{1}{4}$ ppm. In a separate series of experiments (R. D. Blackburn unpublished data) during 1970, it was found that Cutrine applied at an equivalent rate of copper sulfate was less toxic to fish, yet it controlled Saprolegnia. T. Collins and P. Brugeman (personal communication 1970) state that the chelated organic copper compound (Cutrine) is effective in controlling Saprolegnia with no fish kill when applied at the recommended rate of 2.2 ppm/acre.

Willoughby (1968) commented that non-oogonial forms of Saprolegnia usually isolated from fish constitute a definite proportion of the microflora of aquatic areas. The significance of this to the environmental make up of fresh water should not be underestimated. Further adequate methods of control should be developed to prevent the loss of fish populations to help maintain a proper ecological balance. With more research in the physiology of the aquatic Phycomycetes, their relationship to the total ecology should become apparent.

MATERIALS AND METHODS

DESCRIPTION OF THE SAMPLING AREAS

The five sampling areas are located within Pleasant Prairie Township which is located in the Southeast corner of Kenosha County, Wisconsin. Each sampling area is identified on the map of Pleasant Prairie (Fig. 2).

Mahoney's Creek is a very narrow, shallow, meandering creek that flows Eastward from Mahoney's Pond to 34th Avenue where it empties into a storm sewer. Its width varies from two to four feet and the depth varies from six inches to two feet. The bottom is composed primarily of sand, gravel and silt. The vegetation on the creek bank is composed of cattails, Saggetaria, sedges and willows. The water contains an unidentified filamentous mat of algae attached to debris. The creek flows through an area where the banks rise to eight feet in height at the point of entrance into the storm sewer. The sample area is approximately one hundred yards west of the storm sewer. In this area the creek is fifteen inches deep with eight inches of silt on the bottom. It is seven feet below the normal ground level at this point. The water is usually silty and moves rather slowly (Fig. 3).

Mahoney's Pond is located west of 39th Avenue, south of County Road EZ, east of 52nd Avenue, and north of 89th Street. It is a spring-fed pond about 500 yards long and 60 yards wide with an average depth six feet. The pond has a narrow, shallow shore line that drops off rapidly three feet from the shore at the sampling site. The vegetation in this area is composed basically of willows and cattails along the shore.

The water appears to contain a large amount of silt in suspension at all times. The sampling site is located 150 yards west of 39th Avenue on the north side of the pond. The water exhibits no noticeable movement except wind ripples (Fig. 4).

The Des Plaines River is a long, slow-moving shallow river that begins in Somers Township, Kenosha County and flows southeasterly through Kenosha County to eventually empty into the Illinois River near Joliet, Illinois. At the sampling site, the river is normally sixty feet wide and three feet deep at its deepest point. The sampling point was just south of Highway G. This area changes in depth and width whenever there is a measurable amount of rainfall (which complicated the sampling procedures). The bottom of the river is silty. The main vegetation in the area consists of willows and cattails (Fig. 5).

Barnes Creek is a narrow, rather straight creek approximately one-fourth mile long that begins in or near a marsh east of Highway 32 and empties into Lake Michigan. It is located half-way between 116th Street on the south and 104th Street on the north. The creek forms a pond before entering Lake Michigan. The sampling site is located twenty yards east of First Avenue along the south side of the pond. The pond area is 30 yards wide and five feet deep at this point. The bank of the pond is five feet high and covered with poison ivy. The edges of the pond are normally covered with Cladophora; the water is clear, and bottom is sandy. The water moves slowly toward the lake (Fig. 6).

Lake Michigan forms the entire eastern boundary of Pleasant Prairie Township. The sampling site was located twenty yards north of the mouth of Barnes Creek. At this point the lake has a rocky, sandy bottom with almost constant wave action upon the beach. The water contains sediment, usually sand. A sandy beach is directly adjacent to the lake (Fig. 7).

PROCEDURE

Water samples were collected in traps from each area on one to two week intervals from August 13, 1969, to November 18, 1969, and April 25, 1970, to June 12, 1970. A total of 12 water samples were obtained. Traps were usually set out and picked up on weekends. Normally, if there was no growth apparent in the traps, they were left until the next pickup time. Samples from each area were collected to determine which aquatic Oomycetes were present.

Ten small baby food jars were used as collecting chambers. After the jars were sterilized, five were baited with boiled hemp seeds and five were baited with dead flies. Each jar was covered with a three inch by three inch piece of #12 bleached cheese cloth which was secured with a five foot length of heavy cord. This was a modification of the methods used by Patterson (1967) and A. C. Nelson (personal communication 1969).

Before placing the traps in the study site they were filled one-half full of water from the site. The bottles were then placed in the water and inverted so that the open end was in contact with the water of the sampling site. The jars were left at the sampling site for periods ranging from one to two weeks. Immediately after collecting the traps they were placed in the investigators basement (18°C) for continued incubation.

At the time of the first collection period many of the traps were found missing or mouth up. In order to rectify the situation, the investigator devised floats made of 3/8th inch styrofoam cut into four by six inch pieces, Two holes, the diameter of the jars, were cut in each with a hole punched in the end for the cord (Fig. 8 & 9). The floats were placed at each sampling site with one trap containing hemp seeds and the second containing dead flies. Each trap was filled one-half full of water from the site and inverted so the mouths would be in contact with the water. Exeellent results were obtained, except in cases where the water level rose or fell drastically.

Each seed and fly was examined under the microscope at 100X to determine if there was any fungal growth present. If so, each was examined to determine what type of zoosporangial, oogonial and hyphal structures were present in order to identify the organism according to Sparrow (1960) and Gilman (1957).

The investigator tried several stains and fixatives to aid in identification but they caused distortion of the organism and attempts to employ them were unsuccessful.

Collections were not made during the months when each body of water had an ice cover; however, one gallon samples were collected and baited and left at room temperature, along with those from the traps collected in late November. When growth appeared the baits were examined as previously stated to determine the organism present.

In order to obtain pure cultures of the Oomycetes growing on the baits a standard corn meal agar media was prepared by using 17 g. of Difco Corn Meal Agar and 1000 ml of distilled water. This media was sterilized for 15 minutes at 15 lbs pressure. The cooled media was poured into pre-sterilized plastic petri dishes and allowed to solidfy.

Sterile glass slides, three inches by one-half inch, were placed upright into the solidified agar through the center of the plate, dividing it into two equal halves. Each plate was inoculated by centering a small portion of the bait from each trap one-fourth inch in from the slide on one side only. The innoculated plates were allowed to incubate at 21°C to simulate normal water temperatures. When good mycelial growth appeared on the opposite side of the slide from the point of innoculation the mycelial material was aseptically transfered to corn meal agar slants. The slants were allowed to incubate at 21°C until good growth was apparent at which time each culture was examined.

If one is interested in obtaining and maintaining pure cultures, Miller (1967), Wolf (1942), Dick (1965) and Barksdale (1962) have methods which appear to be satisfactory for this purpose.

Figure 1. Developmental stages in the life history of *Saprolegnia* sp.

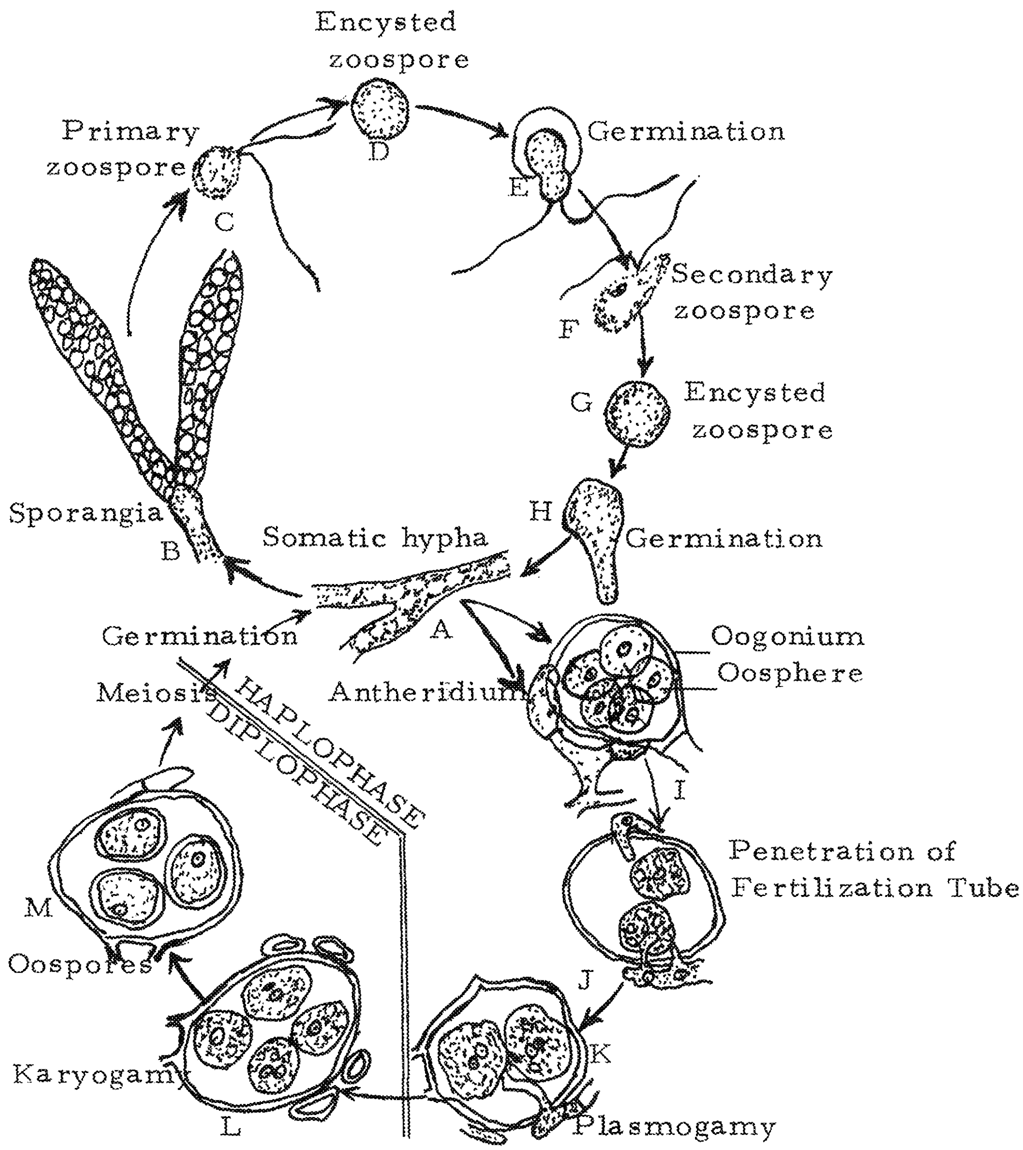
Encysted
zoospore
D
Germination
E
Primary
zoospore
C
Secondary
zoospore
F
G
Encysted
zoospore
H
Germination
Sporangia
B
Somatic hypha
A
Germination
Meiosis
HAPLOPHASE
DIPLOPHASE
Antheridium
Oogonium
Oosphere
I
Penetration of
Fertilization Tube
M
Oospores
J
K
Karyogamy
L
Plasmogamy

Figure 1

Figure 2. Map of Pleasant Prairie Township indicating location of sampling sites

Figure 3. Sampling site on Mahoney's Creek

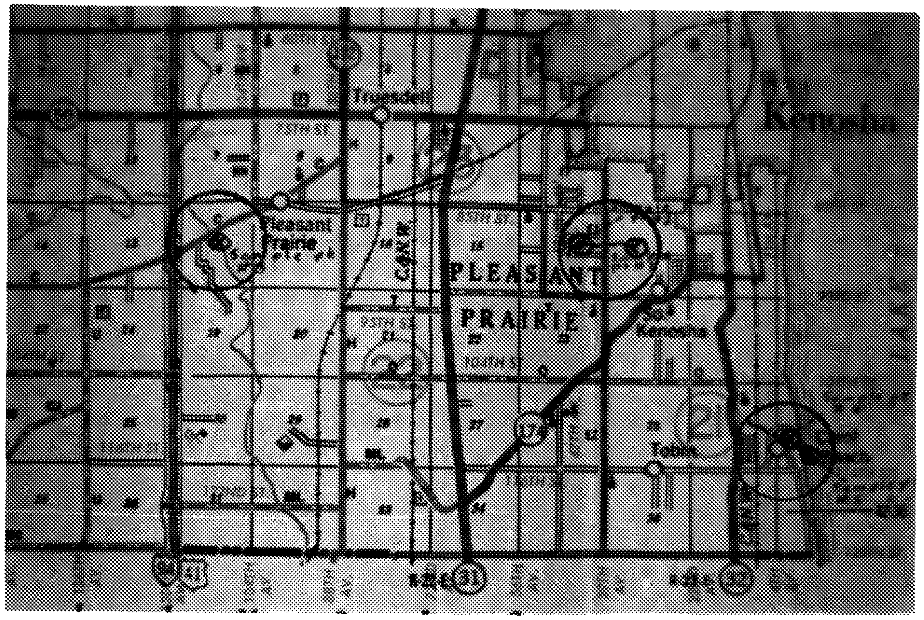

Figure 2

Figure 3

Figure 4. Sampling site on Mahoney's Pond

Figure 5. Sampling site on the Des Plaines River

Figure 4

Figure 5

Figure 6. Sampling site on Barnes Creek

Figure 7. Sampling site on Lake Michigan

Figure 6

Figure 7

Figure 8. Traps used to isolate aquatic Oomycetes

Figure 9. Traps showing placement of covered jars in the styrofoam

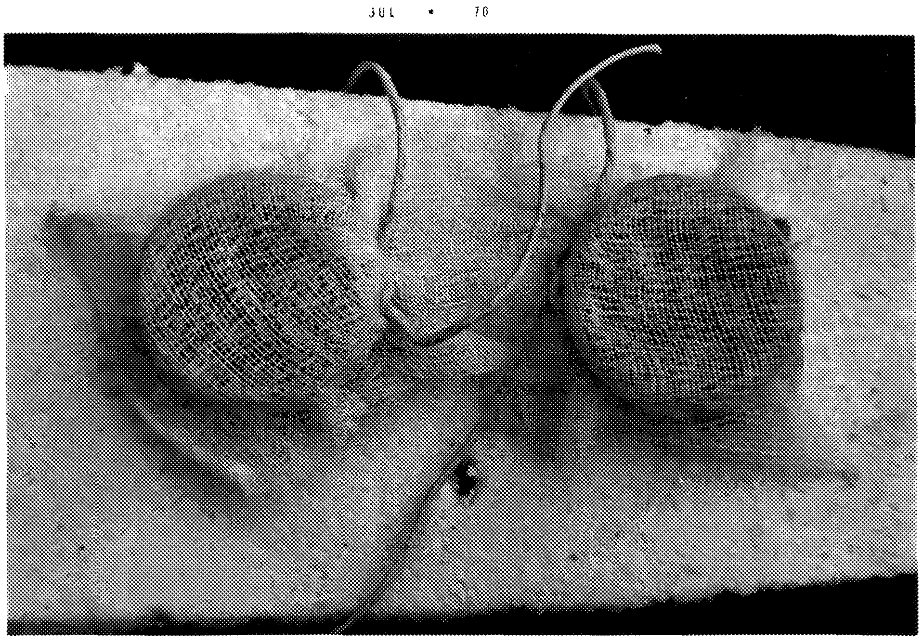

Figure 8

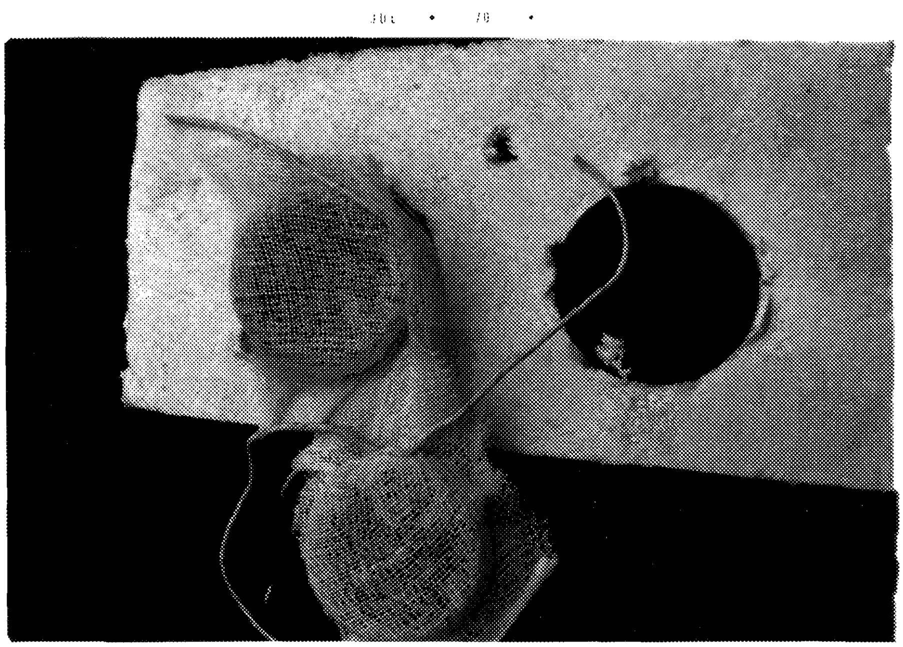

Figure 9

RESULTS

A total of five species of Oomycetes were isolated and identified; a Mucorales and a Chytridiales were also found. Of the five Oomycetes, three were members of the Saprolegniales, one Leptomitales and one Personosporales. The Saprolegniales identified were: Saprolegnia ferax, Saprolegnia diclina, and Dictyuchus sp. The Leptomitales was Apodachyella completa and the Peronosporales was a Pythium sp.

The most frequently isolated and identified species was S. ferax. It was found in all sampling sites, using both flies and hemp seeds, except Barnes Creek. Pythium sp. was identified at two locations. It was found using the flies at Barnes Creek and the hemp seeds at Barnes and Mahoney's Creek (Table 1).

S. diclina was identified, using flies, at three locations--Barnes Creek, Lake Michigan and Des Plaines River. Dictyuchus was identified, using flies, at Mahoney's pond and Lake Michigan. A. completa was identified only at one site. This site was Lake Michigan and hemp seeds were used as bait (Table 1).

The types of Oomycetes did not vary significantly with the location. S. ferax was found in the still waters of Mahoney's Pond, the running waters of Mahoney's Creek and in the turbulent wave action waters of Lake Michigan. It appeared to be universally distributed.

Only two species were isolated from still pond water--Dictyuchus and S. ferax. However, both were also found in Lake Michigan.

SITE	ORGANISM				
	Apodachyella completa	*Saprolegnia ferax*	*Saprolegnia diclina*	*Dictyuchus*	*Pythium*
Mahoney Creek					
Fly		+			+
Hemp		+			
Mahoney Pond					
Fly		+		+	
Hemp		+			
Des Plaines River					
Fly			+		
Hemp		+			
Barnes Creek					
Fly			+		+
Hemp					+
Lake Michigan					
Fly			+	+	
Hemp	+	+			

Table I. Distribution of Oomycetes

Pythium was isolated from the creeks only and not isolated from the rivers, ponds or lake. This species was identified from Barnes and Mahoney's Creek. *S. diclina* and the Mucorales were isolated from both creek water and the lake. *A. completa* was isolated from lake water only.

There did not appear to be any effect on the number of colonies due to seasonal changes nor did there appear to be a major difference in species by season in this study.

The number of species found in each water sample did not differ significantly with the use of either hemp seed or fly as a substratum. There were nine Oomycetes isolated using a fly as a substrate and seven Oomycetes isolated using hemp seeds as a substrate.

Each species was identified by measurement of their vegetative and reproductive organs and associated characteristics as presented in Gilman (1957) and Sparrow (1960). Table 2 contains both the expected and observed characteristics and measurements as a source for comparison.

ORGAN	ORGANISM				
	Saprolegnia ferax	*Saprolegnia diclina*	*Dictyuchus*	*Apodachyella completa*	*Pythium*
Oogonia					
Expected	37 - 97*	35 - 100	____[1]	23 - 48	____[1]
Observed	81.4*	66	____[1]	36	____[1]
Oospore					
Expected	24 - 30	20 - 26	____[1]	18 - 24	____[1]
Observed	25	22	____[1]	18	____[1]
Zoosporangia					
Expected	____[2]	____[2]	14-36x500	____[3]	20 - 30
Observed	37 x 300	30 x 400	25 x 250	____[3]	30
Zoospore					
Expected	9	11 - 11.5	11 - 16	____[3]	8 - 10
Observed	9	11	11 - 13	____[3]	9

* all units are in microns
1 Some species do not show oogonia (Gilman 1957, Sparrow 1960)
2 Sizes not given in Sparrow (1960) or Gilman (1957)
3 *Apodachyella completa* does not have zoosporangia (Sparrow 1960)

Table II. Comparative Reproductive Structures

DISCUSSION

As the purpose of this study was to isolate and identify the Oomycetes found in these five sampling sites in Kenosha County, it is necessary to describe the characteristics by which the species were identified.

Saprolegnia ferax had hyphae that were moderately stout and sparingly branched (Fig. 10, 11). There were many sporangia that were wavy and often tapered upward; the zoospores were generally 9 microns in diameter (Fig. 12). Very few gemmae were found within this species. The oogonia were numerous and varied in size (see Table 1) with walls 1-1.6 microns thick which contained pits. The oogonia were not usually found in chains but were terminal and sometimes intercalary. Oospores were centric and numbered from 1-20. Antheridia were rarely found in this species (Fig. 10,11).

Saprolegnia diclina had moderately sized, little branched hyphae (Fig. 13). Some of the Sporangia were enlarged and broad near the end. Spores were 11 microns in diameter (Fig. 14). S. diclina is diclinous with oogonia and antheridia being produced on the same mycelium (Fig. 15). Oogonia were found in chains and were intercalary as well as terminal. Antheridial branches arose diclinously from near or distant hyphae and sometimes covered the oogonia. Oospores ranged in size (Table 1) and numbered from 2-20.

Vegetatively Dictyuchus is similar in appearance to Achlya. The sporangia were cylindrical, blunt and borne in a zigzag pattern. The sporangia showed a strong tendency to fall away and lie free in the water prior to spore escape. The spores remained in the sporangia forming a network and eventually emerge by individual openings to the outside as secondary zoospores. The oogonia were spherical and smooth when present. Oospores were one to many but didn't fill the oogonia (Fig. 16).

Apodachyella completa was composed of slender, filamentous, irregularly branched mycelia containing pseudosepta. Zoosporangia were not observed in this genera. A. completa differs from Apodachyla by having more than one oospore per oogonia. The oogonium were spherical, terminal and single. The oospores contained a large eccentric oil drop and were approximately 16 microns in diameter.

In Pythium sp. the mycelium was well developed consisting of much branched hyphae which formed tangled complexes. The zoosporangia were filamentous and simple. Zoospores were biflagellated and reniform. Oogonia were not observed .

There were limitations to this study of the Oomycetes. The prime limitation is that this is a taxonomic study only, and no attempt was made to correlate the quantity of Oomycetes with season, temperature, pH or location. The results therefore are valid as organisms were isolated and identified for each location.

A limitation at each location was that only one site was chosen and this particular site was used throughout the study. A more comprehensive study on distribution should have included more sites at each area at spaced intervals to determine Oomycetes over a broader area and changes with location and current in each area.

However, in a study done by Paterson (1967) on the aquatic habitats of Phycomycetes in Northern Michigan, it was found that there was no distribution pattern. It was noted that he found S. ferax and S. diclina among other organisms in Lake Michigan and Douglas Lake using hemp seeds as his most effective bait.

A limiting problem of this study was the character of the water itself. The investigator was hampered many times from collecting samples by the great variation in water level due to rainfall or the lack of it and the turbulence of the water due to wind speed and direction.

A technical problem faced was the use of the stain, Lactophenol-cotton blue. The stain, which was intended for use as a stain and preservative for the organism, instead distorted the shapes of the reproductive organs and made the organism even more difficult to identify.

Figure 10. Mycelium, sporangia and oogonia of *Saprolegnia ferax*, X 61.

Figure 11. Mycelium, sporangia and oogonia of *Saprolegnia ferax*, X 111.

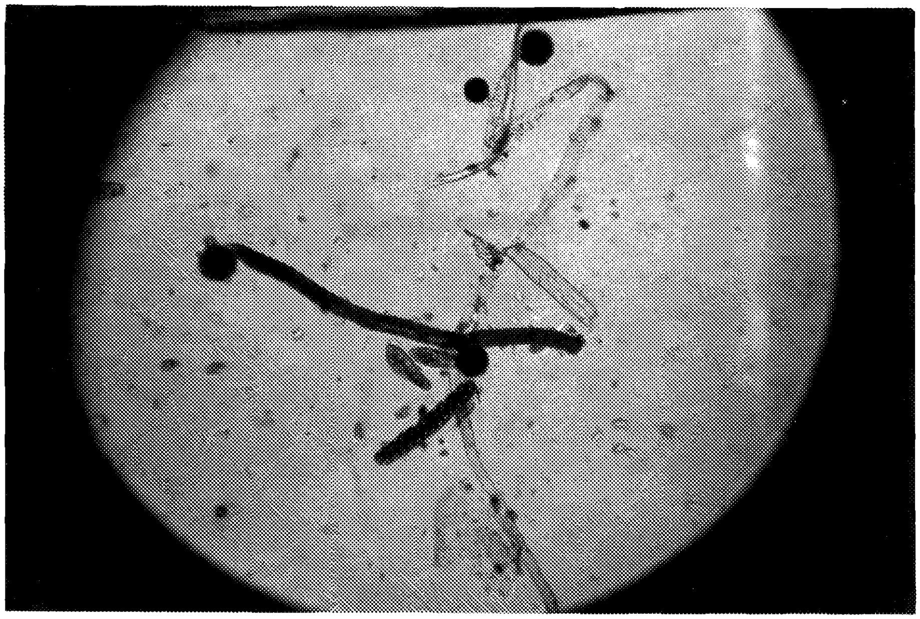

Figure 10

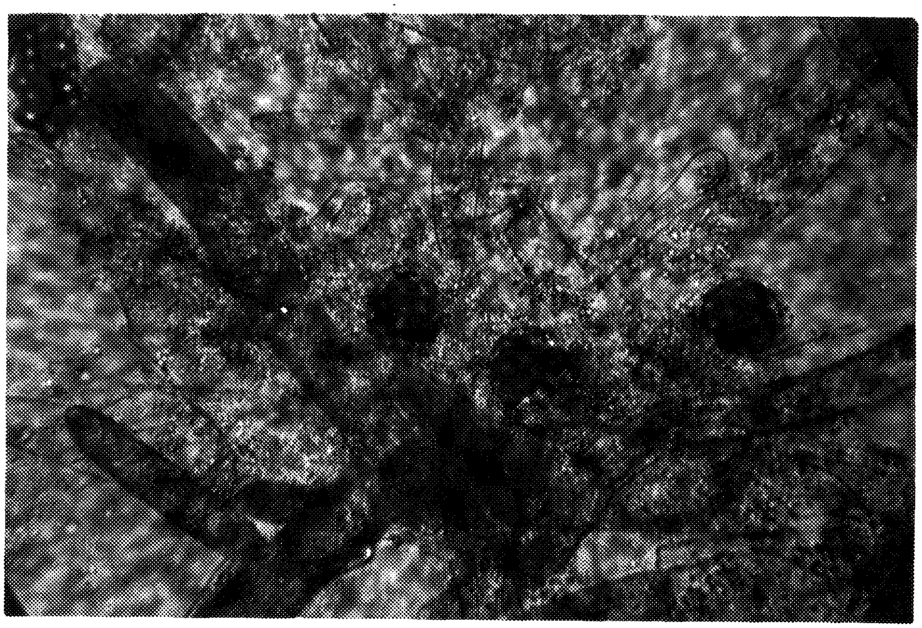

Figure 11

Figure 12. Zoosporangia showing two encysted primary zoospores, X 460.

Figure 13. Mycelium, sporangia and oogonia of *Saprolegnia diclina*, X 75.

Figure 12

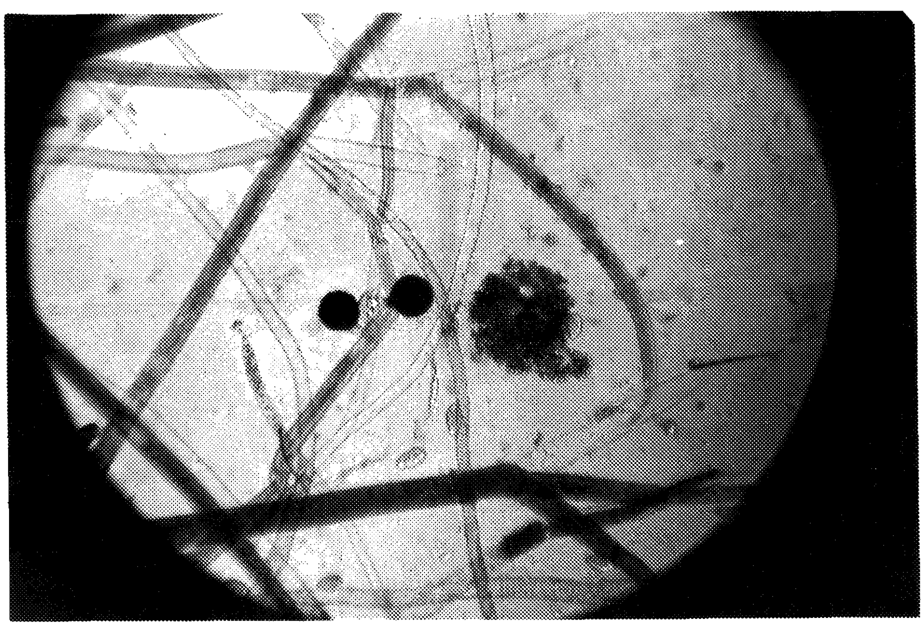

Figure 13

Figure 14. Zoosporangia releasing zoospores of *Saprolegnia diclina*, X 187.

Figure 15. Oogonia with antheridia of *Saprolegnia diclina*, X 220.

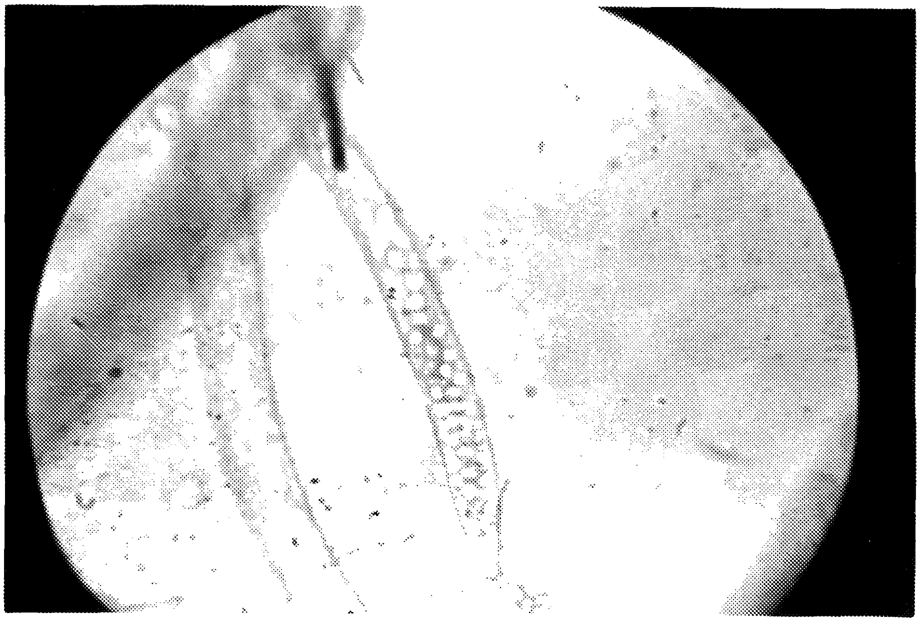

Figure 14

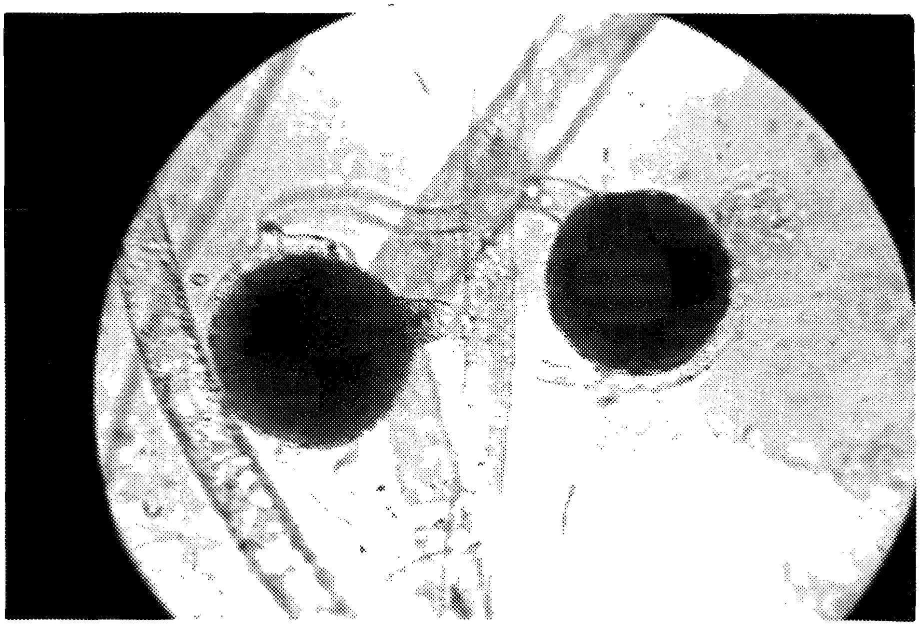

Figure 15

Figure 16. Zoosporangia of Dictyuchus sp. showing zoospores, X 909.

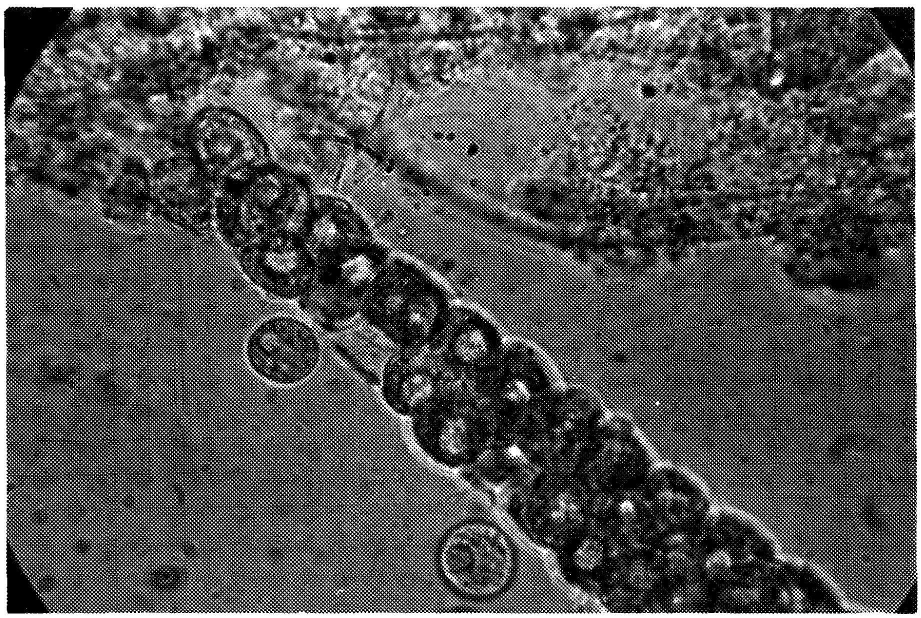

Figure 16

SUMMARY

The purpose of this study was to determine the types of Oomycetes present in aquatic habitats in Pleasant Prairie Township. These organisms were collected with the use of traps and then cultured on agar to obtain pure cultures. With the use of high microscopic magnification reproductive structures could be observed and identification could be made. Pictures and photomicrographs were used in recording sampling sites and illustrating various reproductive structures. The results of this study have established that *Saprolegnia ferax*, *Saprolegnia diclina*, *Apodachyella completa*, *Dictyuchus* sp. and *Pythium* sp. were present.

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