AQUATIC OOMYCETES IN FARM PONDS IN BRYAN COUNTY, OKLAHOMA

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A 2-month preliminary survey of Oömycetes was conducted in 3 farm ponds near Durant, Oklahoma, during the summer of 1973. Ponds were sampled at bi- and tri-weekly intervals and 324 samples were examined for Oomycetes. A total of 322 single-spore pure culture isolations representing 6 genera (*Achlya, Aphanomyces, Saprolegnia, Pythium, Dictyuchus,* and *Leptolegnia*) were made. Dominant genera were *Achlya, Aphanomyces,* and *Pythium*. With the exception of *Saprolegnia,* populations were relatively constant with sampling time, ponds, and locations. *Pythium* and *Aphanomyces* dominated deeper water. Hemp seeds and snake skin were superior to pollen and dead insects as bait for the isolation of these organisms.

INTRODUCTION

Oömycetes, saprobic aquatic fungi readily isolable from fresh water samples, are among the best known aquatic fungi (1). They are commonly known to colonize plant and animal debris in fresh water (2). Although little quantitative information is available, their cosmopolitan distribution and ability to colonize a wide variety of substrates suggest a role in the decomposition of organic materials in fresh water ecosystems. Therefore, elucidation of the occurrence and distribution of these organisms in farm ponds would be indicative of a role in recycling of pond biomass. The present study represents an initial assessment of their importance in farm pond ecosystems. In this study we determined the dominant genera of aquatic Oömycetes during summer months, their distribution both within and between ponds, and the efficiency of various baits for detecting their presence.

Populations of these fungi in 3 farm ponds near Durant, Oklahoma, were examined during the summer of 1973. Work was conducted at the Water Quality Management Laboratory in Durant, Oklahoma, and represents a continuation of an on-going study of the microbiology of farm ponds.

METHODS

Description of the ponds is given in Table 1. Two of the ponds (1a and 1b) are located about ¼ mile apart on the same watershed five miles east of Durant, Oklahoma. Pond 15 is located four miles west of Caddo, Oklahoma. Collections were started on 6 June, 18 June, 11 July and 1 August and generally 2-3 days were required to complete each sampling operation. Samples were collected in the morning and processed in the afternoon. Each pond has a large retaining bank at one end. Water and soil samples were taken from the immediate shore area at three sampling sites in each pond: opposite the retaining bank (site 1), and on the two sides perpendicular to an imaginary line drawn between site 1 and the retaining bank. Each of the ponds was also subdivided into deep (*ca*. 360 cm), intermediate (*ca*. 240 cm), and shallow (*ca*. 120 cm) areas for sampling purposes. A long pole with previously sterilized milk dilution bottles (*ca*. 150 m*l* capacity) attached at *ca*. 30-cm intervals was used to sample water in the deep, intermediate, and shallow areas. Bottles were opened simultaneously by means of a cord and then capped immediately as they broke the water surface. Adjacent samples on the pole were pooled after collection so that the lowermost pooled sample represented water from a depth of 300-360 cm (or 180-240 or 60-120 cm, depending upon the area being sampled). A surface water sample was taken before the pole was placed into position. In addition an Ekman dredge was used to obtain bottom soil samples in each area. Thus, a total of 27 samples was taken from each pond at each sampling time: 24 water samples and 6 soil samples.

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TABLE 1. *Characteristics of farm ponds studied.*

Pond no.	Clarity	Surface area (ha)	Watershed area (ha)	Average depth (m)	Watershed soil type	Watershed vegetation	Grazing
1a	Clear	1.32	10	1.54	Bowie fine sandy loam	Bermuda-grass	Intermittent
1b	Turbid	0.5	10	1.52	Bowie fine sandy loam	Bermuda-grass	Intermittent
15	Clear	1.54	6	1.84	San Saba clay	Bermuda-grass	Continuous

Four different substrates were used for baiting: hemp seeds, snake skin (copperhead), pollen grains (pine), and dead insects (midges and gnats). The pooled water samples were subdivided into four 75-m*l* portions, baited and incubated at ambient room temperature for 2 weeks.

Soil samples were also equally subdivided. Sufficient sterile water (a one-to-one mixture of pond water and distilled water) was added to cover the 5-10 g of soil used. Baits were added to the samples and the mixtures incubated at room temperature for two weeks. When aquatic fungi became visible on the baits, they were transferred to corn meal agar containing penicillin (300 units/m*l*) and streptomycin (300 μg/m*l*). Agar blocks were cut after the hyphae were established, transferred to sterile water, and incubated until zoospores were released. Pure cultures were established by isolating single zoospores or single hyphal tips. Generic identification was made from the pure cultures.

RESULTS

A total of 324 samples were collected and subdivided for comparison of baits, for a total of 1053 subsamples. If water molds were isolated from any or all of the baits from a sample, the sample was scored as positive for Oömycetes. Of the 324 samples, 61.8% yielded at least one genus of Oömycetes, and 74.2%, 71.3% and 39.8% of the samples from ponds 1a, 1b, and 15, respectively, were positive for these fungi. Individual positive samples frequently yielded as many as 3-4 genera.

The following genera of Oömycetes were isolated in the study: *Achlya, Saprolegnia, Pythium, Aphanomyces, Dictyuchus,* and *Leptolegnia.* Chytridiomycetes and Leptomitales were frequently observed but not identified since they were beyond the scope of this preliminary investigation. The most frequently isolated fungi were *Achlya* and *Aphanomyces,* each of which were isolated from approximately 33% of the samples. Table 2 summarizes the number of isolations of each genus from each pond. Pond 15 appeared to support a smaller population of these fungi than the other ponds. In addition, the relative number of *Achlya* isolations was much lower and the relative number of *Aphanomyces* and *Pythium* isolations was much higher.

TABLE 2. *Number of isolations of Oömycetes.*

Pond	Sampling date	*Achlya*	*Aphanomyces*	*Saprolegnia*	*Pythium*	*Dictyuchus*	*Leptolegnia*	Total
	6/6	9	5	8	4	2	0	28
	6/20	8	15	5	7	2	0	37
1A	7/11	23	15	0	5	2	1	46
(turbid)		10	15	1	8	3	0	37
subtotal	8/1	50	50	14	24	9	1	148
	6/7	4	5	10	8	2	1	30
	6/22	13	8	6	6	2	0	35
1B	7/13	15	12	0	1	1	0	29
(clear)		14	10	0	4	0	0	28
subtotal	8/1	46	35	16	19	5	1	122
	6/6	0	2	4	4	0	0	10
	6/18	2	2	0	9	1	0	14
15	7/9	3	11	0	2	0	0	16
(clear)	7/31	4	6	0	2	0	0	12
subtotal		9	21	4	17	1	0	52
Total		105	106	34	60	15	2	322

TABLE 3. *Number of isolates from different areas of the ponds.*

		Achlya	*Aphanomyces*	*Saprolegnia*	*Pythium*	*Dictyuchus*	*Leptolegnia*	Total	Isolations per sample
Shore areas	Surface water	26	16	12	12	0	0	66	1.8
	shore soil	17	17	9	11	4	0	58	1.6
Shallow areas	surface	3	5	1	2	0	1	12	
	0-30 cm	5	5	1	2	2	0	15	
	30-60 cm	4	2	2	0	0	0	8	
	bottom water[a]	5	5	2	2	2	1	17	
	bottom soil	0	1	0	5	0	0	6	
	subtotal	17	18	6	11	4	2	58	1.0
Intermediate areas	surface	7	5	3	1	0	0	16	
	0-60 cm	2	2	1	0	1	0	6	
	60-120 cm	3	2	0	1	1	0	7	
	120-180 cm	5	6	1	0	1	0	13	
	180-240 cm	4	6	0	1	1	0	12	
	bottom water[a]	1	4	0	3	1	0	9	
	bottom soil	0	1	0	2	0	0	3	
	subtotal	22	26	5	8	5	0	66	0.8
Deep areas	surface	3	5	2	3	0	0	13	
	0-60 cm	5	5	0	0	0	0	10	
	60-120 cm	2	5	0	2	0	0	9	
	120-180 cm	4	6	0	0	0	0	10	
	180-240 cm	4	3	0	1	0	0	8	
	240-300 cm	1	1	0	0	0	0	2	
	300-360 cm	3	3	0	2	0	0	8	
	bottom water[a]	1	1	0	6	2	0	10	
	bottom soil	0	0	0	4	0	0	4	
	subtotal	23	29	2	18	2	0	74	0.7
	total	105	106	34	60	15	2	322	

[a]Water at mud-water interface.

TABLE 4. *Comparison of baits for isolation of Oömycetes.*

Bait	*Achlya*	*Aphano-myces*	*Sapro-legnia*	*Pythium*	*Dicty-uchus*	*Lepto-legnia*	Total Isolations	Total samples	Isolations per sample
Hempseed	81 (42.6%)	53 (27.9%)	20 (10.5%)	27 (14.2%)	9 (4.7%)	0 --	190	324	0.6
Insect	44 (37.6%)	34 (29.1%)	17 (14.5%)	14 (12.0%)	7 (6.0%)	1 (0.9%)	117	243	0.5
Snake skin	18 (19.8%)	38 (41.8%)	12 (13.2%)	21 (23.1%)	0 --	1 (1.1%)	91	324	0.3
Pollen	2 (6.9%)	12 (41.4%)	4 (13.8%)	11 (37.9%)	0 --	0 --	29	162	0.2

Ponds la and lb had a very similar Oömycetes flora. In addition, the number of total Oömycete isolations in each pond was nearly constant over the course of the study. On the other hand, *Saprolegnia* was isolated much more frequently in early June than in the later collections and a higher number of *Achlya* isolations were made in late June and July than in early June. Because of the general similarity in numbers of isolations between the various sampling dates, the sampling time data were pooled in Tables 3 and 4. The number of Oömycete isolations, except for *Dictyuchus*, from the shore areas and the different banks and shore soil of the ponds were generally similar (Table 3). This table also summarizes the number of isolations of each genus from the various pond areas and represents a composite of all 3 ponds. The shore areas yielded an average of 1.7 isolations per sample (1.8 and 1.6 for water and soil, respectively) whereas a progressive decrease in isolations per sample was noted as more samples from deeper water were included (Table 3).

Although the deeper areas were less productive than the shore ones, deeper areas yielded isolates of all the genera reported. In general, organisms were less frequently isolated in deeper water than in shallow water, although *Pythium* was more frequently isolated from the bottom water and bottom soil than from the upper levels of the pond. Whereas *Pythium* isolations accounted for 18.6% (60/322) of the total isolations, they accounted for 31% (11/36) of total bottom water and 85% (11/13) of the total bottom soil isolations. *Aphanomyces* was frequently isolated from bottom water and only *Pythium* and *Aphanomyces* were isolated from bottom soil (Table 3).

The comparison of baits for Oömycetes isolation is shown in Table 4. Although all 4 baits were not used with every sample because of time limitations, hemp seeds and snake skin were used with all samples. Insect bait was used with 3 of the 4 sets of samples, and pollen for 2 of the sets of samples. Since the number of samples baited is not constant for each of the baits, it is perhaps more meaningful to compare the average number of isolations per sample. Thus for the Oömycetes recorded hemp seeds were superior to the other baits and pollen was the least effective. In general, isolation frequency for each of the genera was similar for the hemp seeds and insects although bacterial contamination was much greater with the insect bait. This pattern was markedly different when snake skin or pollen were used as bait (Table 4). *Achlya* was the dominant genus isolated from hemp seeds or insect bait but *Aphanomyces* was the dominant genus on snake skin and *Achlya* isolations were much less frequent. *Achlya* spp. were infrequently isolated from pollen while *Aphanomyces* and *Pythium* were the dominant genera isolated. Although 61.8% of the pooled samples yielded at least one water mold, only 427 isolations were made from the 1053 sample units. In many instances, organisms were isolated from only one of the four baits employed; thus it is desirable to use more than one bait in studies of this type. Because of the similarity of isolation patterns between insect bait and hemp seeds, the use of insect bait is not recommended. Pollen was useful for isolation of Chytridiomycetes as well as in that of *Aphanomyces* and *Pythium.*

Selected chemical properties of the three

TABLE 5. *Chemical properties of pond water and mud (average of observations in March 1973 and March 1974).*

Property	Pond	Mud	Water at mud-water interface	Water column
pH	1a	5.3	7.2	7.47
	1b	5.2	6.5	7.23
	15	7.5	7.8	8.21
Conductance (mho/cm)	1a		98.5	115.43
	1b		34.9	52.34
	15		255.0	252.43
		(mg/kg)		
Organic carbon	1a	27,900		
	1b	15,600		
	15	30,400		
			(mg/*l*)	(mg/*l*)
Total N	1a	2,468	21.92	0.66
	1b	1,678	62.45	0.61
	15	3,823	1.00	0.79
NO_3—N	1a	21.8	0.382	0.17
	1b	11.6	0.216	0.12
	15	16.3	0.451	0.44
NH_4—N	1a	106.4	0.080	0.06
	1b	64.9	0.089	0.08
	15	137.5	0.257	0.23
NO_2—N	1a		0.004	0.01
	1b		0.004	0.01
	15		0.009	0.01
Total P	1a	340.0	4.439	0.061
	1b	250.0	1.522	0.109
	15	1,135.0	0.043	0.034
Total water-soluble P	1a		0.021	0.026
	1b		0.015	0.028
	15		0.008	0.010
Ortho P passing 0.45 filter	1a		0.005	0.003
	1b		0.002	0.003
	15		0.006	0.005
Alkalinity (as $CaCO_3$)	1a		65.8	64.09
	1b		47.5	34.95
	15		116.8	108.98
Chloride	1a		12.1	10.46
	1b		5.3	4.76
	15		9.2	10.81
Calcium	1a		9.3	13.14
	1b		2.8	5.77
	15		18.2	18.34
Magnesium	1a		2.01	2.90
	1b		3.01	1.40
	15		2.43	2.42
Potassium	1a		4.6	4.14
	1b		4.9	3.71
	15		3.4	3.30

[a]Water was sampled at 30-cm intervals between 30 Cm and 360 cm from pond bottom inclusive. There was no significant difference between depth intervals in any pond. Values given are means of all intervals. Two locations per pond, each on 360-cm depth contour, were analyzed.

ponds are shown in Table 5. These values are averages of samples taken the spring before and the spring after the present study. Since there was little variation between the two years we have assumed these values to be approximate for the study period as well.

DISCUSSION

Useful correlations could not be made between temperature and the occurrence of Oömycetes in this study. This was probably because the study extended over only 2 months of essentially constant climatic conditions (summer) and because there was more variation in temperature within each sampling time than between sampling times and ponds. Milovtsova (3), in a study of the saprolegniaceous fungi of the Kharkov region of the Soviet Union, reported that *Saprolegnia* tended to predominate in spring samples, whereas *Achlya* was predominant in summer samples. Ziegler (4) and Hughes (5), in separate studies of the southeastern United States, listed all of the species of *Saprolegnia* and *Isoachlya* as "cold weather" fungi or as predominating in "winter samples." The greater occurrence of *Saprolegnia* in our first samples and its virtual disappearance in later samples is consistent with these reports and suggests that early June is the end of its active season in these ponds.

Pythium and *Aphanomyces* were dominant in the deeper water and bottom soil whereas *Achlya* and *Saprolegnia* were dominant in surface water and at the edges of the ponds. In general the occurrence of these fungi in pond 15 resembled that of the deeper water of ponds 1a and 1b.

Chemical data for the ponds show that pond 15, which yielded a much lower number of isolates than ponds 1a and 1b, had a much higher level of ammonia N at all depths. The ammonia concentration of this pond would not be an inhibitory factor since 1) the absolute concentrations are lower than those used in culture media and 2) ammonium salts are excellent sources of nitrogen for these fungi (6). Possibly the lower yield of Oömycetes in pond 15 was influenced by the higher bacterial numbers in this pond (7).

Facultative anaerobic bacteria were more numerous near the surface of pond 15 than in pond 1a or 1b, but not at lower depths. Aerobic counts, however, were consistently higher than anaerobic ones in all ponds, indicating the ponds did not become extremely anaerobic. This was true even in the sediment (7).

It would be interesting to compare direct measurements of O_2 tension in these ponds. The organic carbon was higher in the mud of pond 15 (3.04% compared with 2.79% for pond 1a and 1.56% for pond 1b). Bacterial populations were 10-100 times as high in pond 15 as in the other two ponds during this period, but this was not the case throughout the year (7). Pond 15, on a soil underlain with a limestone formation, also had a higher pH (7.5 in the mud, increasing to 8.4 at the surface, versus 5.2 and 5.3 in the mud, increasing to 7.6 and 7.8 at the surface, for 1b and 1a). Watersoluble P was also low in pond 15 although nonparticulate orthophosphate (that passing a 0.45 μm filter) was slightly higher. Conductance, calcium, and alkalinity were higher in pond 15.

Although all 4 baits were not used with every sample, the same combination of baits was used for all samples taken at a given sampling time, as already described. Since insect bait was so similar to hemp seeds and pollen had a low Oömycete yield these omissions should not have affected the results and in any case would not affect comparison within sampling periods. As noted earlier (Table 2), no notable differences were observed between different sampling times except for the decrease in numbers of *Saprolegnia* isolations. As previously reported by Scott (8) snake skin was especially favorable for the isolation of *Aphanomyces.*

The greater occurrence of water molds at the shore areas of these ponds agrees with previous reports and appears to be generally true for aquatic fungi. Willoughby (9) reported that lake margins are "exceptionally favorable" for the growth of saprophytic chytrids and in 1962 (10) reported much higher numbers of Saprolegniales from the lake margin than from surface waters from the center of the lake. Willoughby and Collins (11) suggest that "the Saprolegniaceous spora in open water receives local reinforcement as the result of fungal activity at the lake margin . . . ."

The ubiquitous presence of one or more genera of Oömycetes indicates to us that these organisms do have a role in biological recycling in farm ponds. In addition, their affinity for baits containing less easily decomposed material such as chitin and cellulose probably indicates a specialized role for these organisms. Their relative abundance on the shore line and in shallow water as opposed to mud and deep water would indicate a greater abundance of energy materials in the littoral zone than in the deeper water or in the sediment.

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