4-1-1980

Life Cycle, Ecology, And Management Considerations Of The Green Filamentous Alga, Pithophora

C. A. Lembi

N. L. Pearlmutter

D. F. Spencer

TECHNICAL REPORT NO. 130

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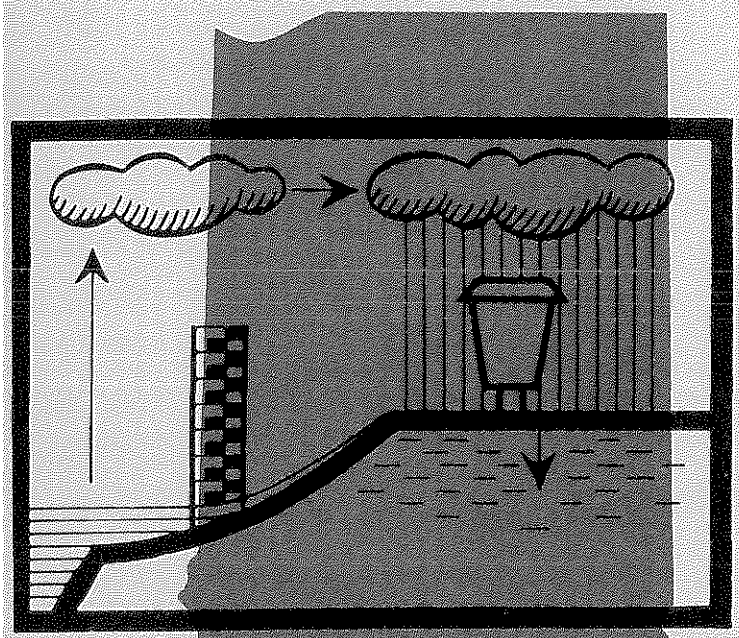
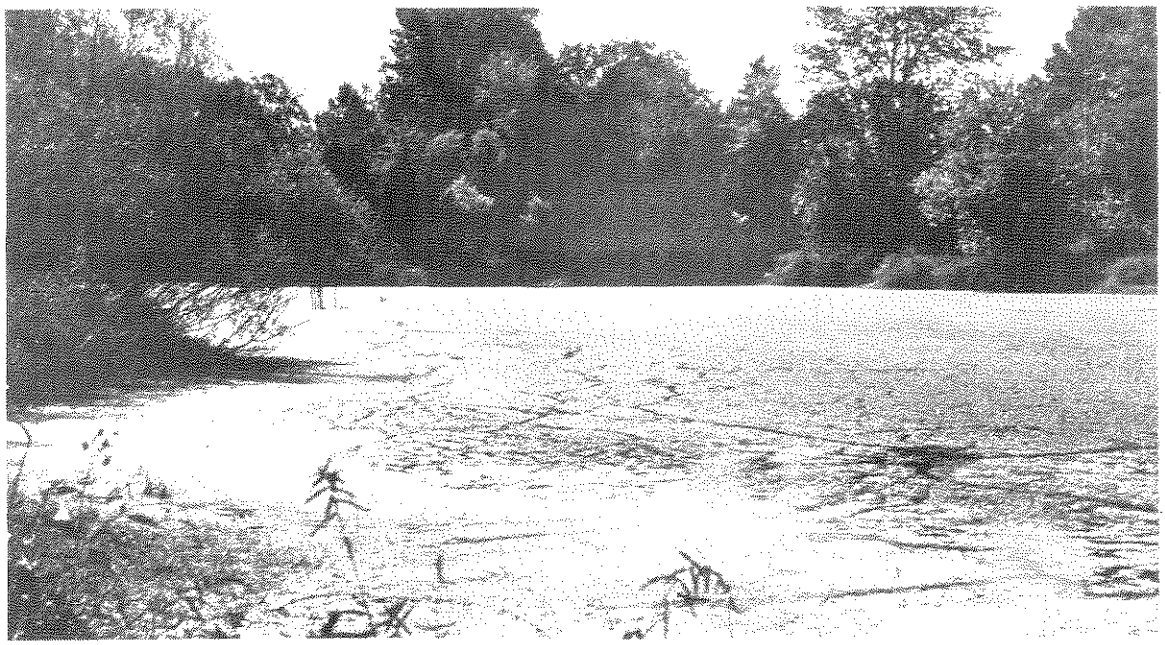


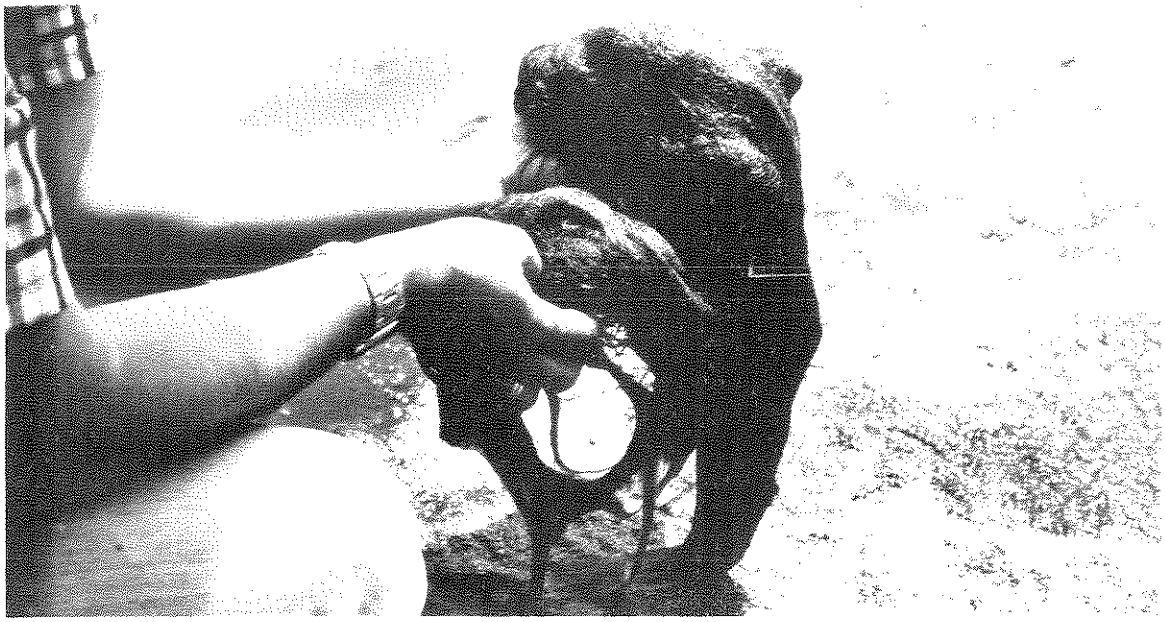
by

Carole A. Lembi
Nina L. Pearlmutter
David F. Spencer

April 1980

PURDUE UNIVERSITY
WATER RESOURCES RESEARCH CENTER
WEST LAFAYETTE, INDIANA





Pithophora in Surrey Lake, Indiana

". . . within the past 5 years, several hundred farm fishponds have become infested with a branched form of filamentous alga that is called Pithophora. Once Pithophora becomes established, it forms a heavy blanket of floating alga over much of the pond surface and thus renders a pond unfit for fishing. In experiments at Auburn, this alga has reduced fish production by as much as 50% by competing with the phytoplankton for plant nutrients. . . ."

Lawrence (1954) in reference to southeastern United States

"Few if any herbicides kill Pithophora and give desirable control".

Crance (1974)

Water Resources Research Center

Purdue University

West Lafayette, Indiana

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The work upon which this report is based was supported in part by funds provided by the United States Department of the Interior, Office of Water Research and Technology, as authorized by the Water Resources Research Act of 1964 (PL 88-379) as amended.

Completion Report for OWRT B-081-IND

Purdue University Water Resources Research Center

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ACKNOWLEDGEMENTS

We would like to acknowledge the excellent technical assistance of Ted R. Volpp whose help and encouragement at crucial points during this study are much appreciated. Also contributing substantial technical assistance were Edward J. King, Kristine Vollmer, and Ruth Gyure. We would like to thank Mr. Bob Weber and Mr. Mike Long of Columbus, Indiana; the former for allowing us to work unhindered at Surrey Lake and the latter for his jovial assistance and encouragement. A special thanks go to the members of the Syracuse Lake Association, Inc., in particular, David W. Stoops and Frank and Virginia Putt, for first alerting us to the Pithophora problem in northeastern Indiana. Nitrogen and phosphorus analyses of the water samples were conducted in the laboratory of Drs. D. W. Nelson and L. E. Sommers. Finally, we acknowledge the support given to this project by Dr. Daniel Wiersma, Director of the Indiana Water Resources Center and by Drs. Mark L. Tomes and T. K. Hodges, former and current heads of the Department of Botany and Plant Pathology, respectively.

Project personnel were Carole A. Lembi, Associate Professor, Purdue University; Nina L. Pearlmutter, Research Assistant (currently Assistant Professor at Iowa State University), and David F. Spencer, Postdoctoral Associate (currently Assistant Professor at I.U.P.U. at Indianapolis).

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ABSTRACT

Pithophora, a green filamentous alga in the order Cladophorales, forms infestations of thick, free-floating mats in small impoundments, shallow lakes, and coves and channels of larger lakes and reservoirs throughout the midwest and southeastern United States. In addition to severely restricting recreational and commercial uses (e.g., catfish farming operations), the organism has a reputation for being extremely difficult to control, in part because of its resistance to presently labeled aquatic herbicides. Most of the research on the ecology and management of littoral filamentous algae has been conducted on its close relative, *Cladophora*. Thus, the purpose of this project was to obtain much needed data on the biology of *Pithophora* in order to establish a basis for management recommendations.

The life cycle of *Pithophora oedogonia* consists of two stages: branching filamentous cells, and akinetes ("spores"). Both cell types are enclosed by a thick cell wall composed primarily of cellulose with additional amounts of sugars, amino sugars, and proteins, the latter being concentrated in the outer cell wall layer. Chitin (the component of insect exoskeletons) comprises 6% of the cell wall by weight and is localized in the outer wall layers, cross wall disks, and associated bands. Its distribution probably accounts for both the distinctive branching pattern and the coarse texture of this organism when compared to other filamentous green algae. Akinetes are more resistant to the algaecide copper sulfate than filamentous cells. Copper is reversibly bound to the outer layer of the cell wall where it appears to exchange with Ca^{++}, Mg^{++}, and Zn^{++} ions; however, the exact chemistry of the binding site is unknown.

Field studies on *P. oedogonia* were conducted in Surrey Lake, a shallow 3.9 ha impoundment in central Indiana. Akinetes form in the free-floating mats in the fall, reach their highest numbers in the winter, and germinate in the spring in response to an increase in water temperature to 20 C. Vegetative biomass is highest in the summer but significant amounts are present throughout the winter under the ice cover. Akinete formation is probably due to nitrogen depletion rather than to increasing periods of cold or declining light intensities. Akinete numbers are high in the hydrosoil ($\bar{X}$ = 11.9 X 10^6 akinetes m^{-2} in the uppermost cm of soil) and show excellent viability to hydrosoil depths of 10 cm. Although specific testing and more

information are required, particularly on the role that hydrosoil akinetes play in the reestablishment of infestations, treatment of the free-floating mats with copper sulfate at a period of low akinete number and biomass (i.e., at or immediately following spring germination at 20 C) should be considered when planning management strategies.

Akinetes show excellent survival when exposed to winter drawdown conditions in the field. They are very susceptible to desiccation, but are protected from the drying effects of summer drawdowns by the thickness of the vegetative mats (which in turn are protected by a surface layer of dead filaments) and by the frequency of rains in the summer. In addition, akinetes appear to form in large numbers as a response to gradual desiccation. Unless the mats and hydrosoil can be disrupted so that the akinetes are exposed to ambient atmospheric conditions, drawdown does not appear to be a viable management alternative.

Nitrogen and phosphorus requirements for vegetative growth at 20 C were determined under laboratory conditions. The half saturation constants relating growth to external nutrient concentrations were 1.23 mg l^{-1} (= 88 μM) for nitrate-N and 0.1 mg l^{-1} (= 3.22 μM) for phosphate-P. The spatial distribution of Pithophora in Surrey Lake was regulated by nitrate-N, but nutrient data from 27 Indiana lakes suggest that phosphate-P concentrations are generally lower than the level required for Pithophora growth and are thus likely responsible for limiting the distribution of this alga in Indiana. We suggest that where feasible, management efforts include the reduction of N and/or P concentrations to levels below the half saturation constants.

INTRODUCTION

Pithophora oedogonia is a green, filamentous alga in the order Cladophorales (Chlorophyceae). It is a free-floating organism forming thick, heavy mats that can completely fill in small impoundments, shallow lakes, and coves and channels of larger lakes and reservoirs. Much of the research emphasis in filamentous algal ecology and management in the past 10-15 years has been placed on its close relative *Cladophora* whose presence and spread in streams and rivers and in the littoral areas of the Great Lakes has been associated with the increased eutrophication of these bodies of water. *Pithophora*, on the other hand, has been virtually ignored although it is generally acknowledged to be a severe management problem in fish-rearing facilities, farm ponds, and shallow lakes throughout the southeastern and midwestern sections of the United States. At the time this project was initiated (1976), we knew only that *Pithophora* was a serious problem in the largest natural lake system in Indiana as well as in numerous farm ponds throughout the state, that it was difficult to manage because of an apparent resistance to aquatic herbicides, and that it formed akinetes (spores) which were a possible cause for the herbicide resistance.

Specific data on the life cycle of the organism, the environmental parameters regulating that life cycle, and the basis for herbicide resistance if resistance does indeed exist is required before successful management methods can be developed. Potential management tools include chemical treatments with herbicides such as copper sulfate, mechanical manipulations such as drawdowns, and the alteration of growth patterns through nutrient limitation. The research reported here is a first step toward developing this data and is divided into the following sections: 1.) the biology and geographic distribution of *Pithophora*, 2.) the effect of copper on the organism, 3.) the life cycle of *Pithophora* in the field with emphasis on akinete periodicity and tolerance to stress conditions, 4.) nitrogen and phosphorus requirements for vegetative growth, and 5.) preliminary findings concerning management approaches.

I. BIOLOGY AND GEOGRAPHIC DISTRIBUTION OF *PITHOPHORA*

Thallus Structure and Life Cycle

Pithophora Wittrock 1877 is placed in the green algal order Cladophorales because it consists of multinucleate cylindrical cells united end to end in simple (e.g., *Basicladia* and *Rhizoclonium*) or branched (*Pithophora* and *Cladophora*) filaments. Each cell is enclosed by a thickened stratified cell wall and contains a reticulate chloroplast with numerous pyrenoids.

As indicated previously, most of the research on the members of this order has been directed toward the biology and ecology of *Cladophora* Kuetzing 1843 and has resulted in numerous scientific articles and several recent monographs on the ecology and nutrition of *Cladophora* found in the Great Lakes (e.g., Gerloff and Fitzgerald, 1976; International Joint Commission, 1975). In contrast, very little is known about *Pithophora*. Reports on the similarities and differences between the two genera are limited to rather general descriptions of morphology and habitat.

As a basis for comparison, Prescott's (1962) description of *Cladophora* is as follows: "A repeatedly branched filamentous thallus with basal-distal differentiation; attached when young, but in some species, becoming free-floating; forming feathery tufts on substrates, especially in flowing water,... asexual reproduction by zoospores; sexual reproduction by isogametes produced in apical or subapical, unspecialized cells."

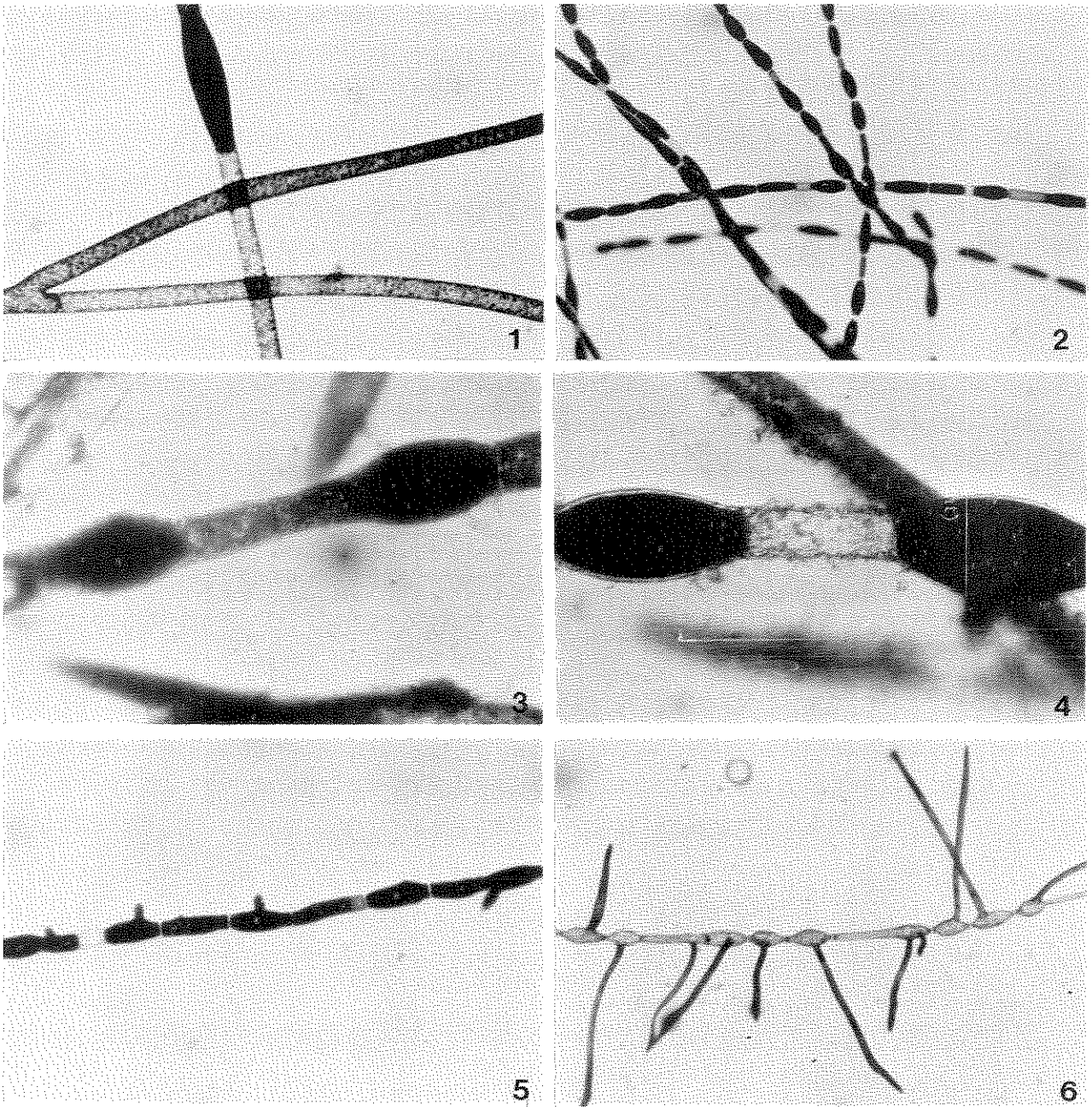
The morphological differences between this genus and *Pithophora* are significant because they explain in part the reason for the wider geographic distribution (in the U.S.) of *Cladophora*. *Pithophora* is branched and filamentous as is *Cladophora*, but in most species, the basal-distal differentiation is lacking. There is no elaboration of rhizoids, a system of prostrate colorless cells originating from a basal cell to anchor the alga to a substrate. Thus, *Pithophora* is a free-floating organism throughout its life cycle and is restricted to areas where water flow is not rapid enough to wash the organism away, i.e., in small ponds or lakes and in the protected coves, bays, and channels of larger lakes. Many of the *Cladophoras*, on the other hand, (in particular the major problem species, *C. glomerata*) are usually attached to a substrate such as rocks and are found in rapidly flowing streams and channels and along the unprotected edges of larger lakes. *Cladophora* often becomes separated from its substrate because of wind or wave action to

form free-floating mats although in some species the vegetation may begin to die and decompose following detachment.

Pithophora does not form motile cells for either asexual or sexual reproduction as does Cladophora. The only two components of the Pithophora life cycle are the long (100-3000 μm), branching filamentous cells and the spores or akinetes which are borne either singly (Fig. 1) or in chains on the filaments (Fig. 2) and which are thought to be densely packed with reserve foods. Akinete formation occurs by the gradual migration of the protoplasmic contents to the upper or distal end of a filamentous cell (Fig. 3). This is followed by the deposition of a cross wall which separates the akinete from the lower portion of the cell (Fig. 4). The purpose of the akinete has been variously proposed as providing the organism with a means of overwintering, surviving desiccation when mats are stranded above the shoreline (Fritsch, 1907) and surviving conditions of nutrient depletion (Ernst, 1908). Although Cladophora glomerata has been reported to form akinetes, primarily in the winter (Bellis and McLarty, 1967; Mason, 1965), their appearance in the genus is relatively rare (Patel 1971; Smith, 1950) whereas akinetes are present to some degree all year in Pithophora and are used as the major identifying characteristic for the genus.

Akinete germination occurs by the extension of the akinete wall to form a germination tube (Fig. 5). Most of the cell contents move into the germination tube which continues to elongate to form a new filamentous cell (Fig. 6). A cross wall forms at the base of the new cell soon after germination and further vegetative growth takes place by cell division of the new filament. The germination tube almost always forms at right angles to the longitudinal wall of the akinete but may also grow through the cross wall.

An additional major difference between Cladophora and Pithophora is the branching pattern of the filaments. Cladophora branches are initiated by an extension of the cell wall at the cross wall. Growth of the lateral branch often displaces the main axis giving the filament a forked appearance (see diagram, Fig. 15A). Branches in Pithophora are initiated some distance (often as much as 10-20 μm) below the cross wall. Therefore, the lateral branch does not displace the main axis (Fig. 15B) and sometimes even appears to arise at right angles to the main axis.



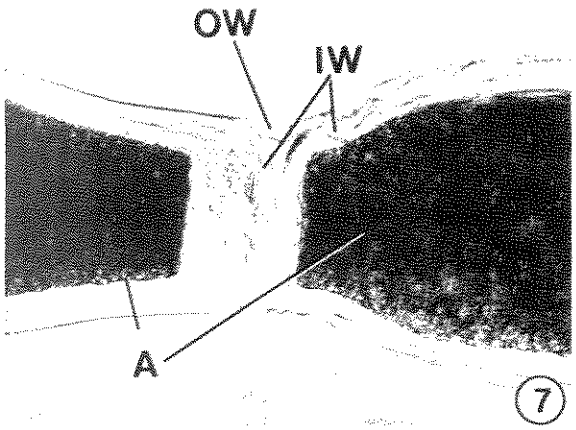
Figs. 1-6: Fig. 1. Akinete and branching filament of Pithophora oedogonia. X 111. 2. Akinete chains. X 22. 3. Akinete formation as cytoplasm moves toward distal end of the cell. X 138. 4. Akinete formation nearly completed. X 166. 5. Initiation of akinete germination. X 55. 6. Akinete germination with elongating germination tubes. X 39.

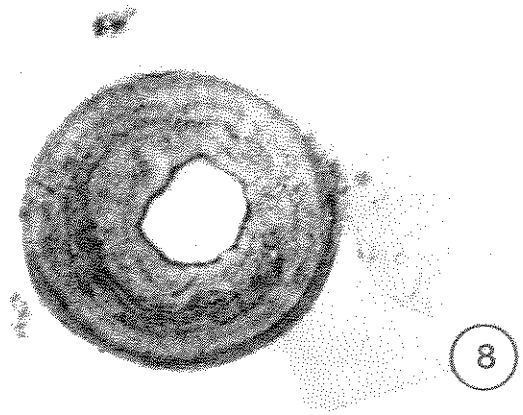
Cell Wall Structure and Composition

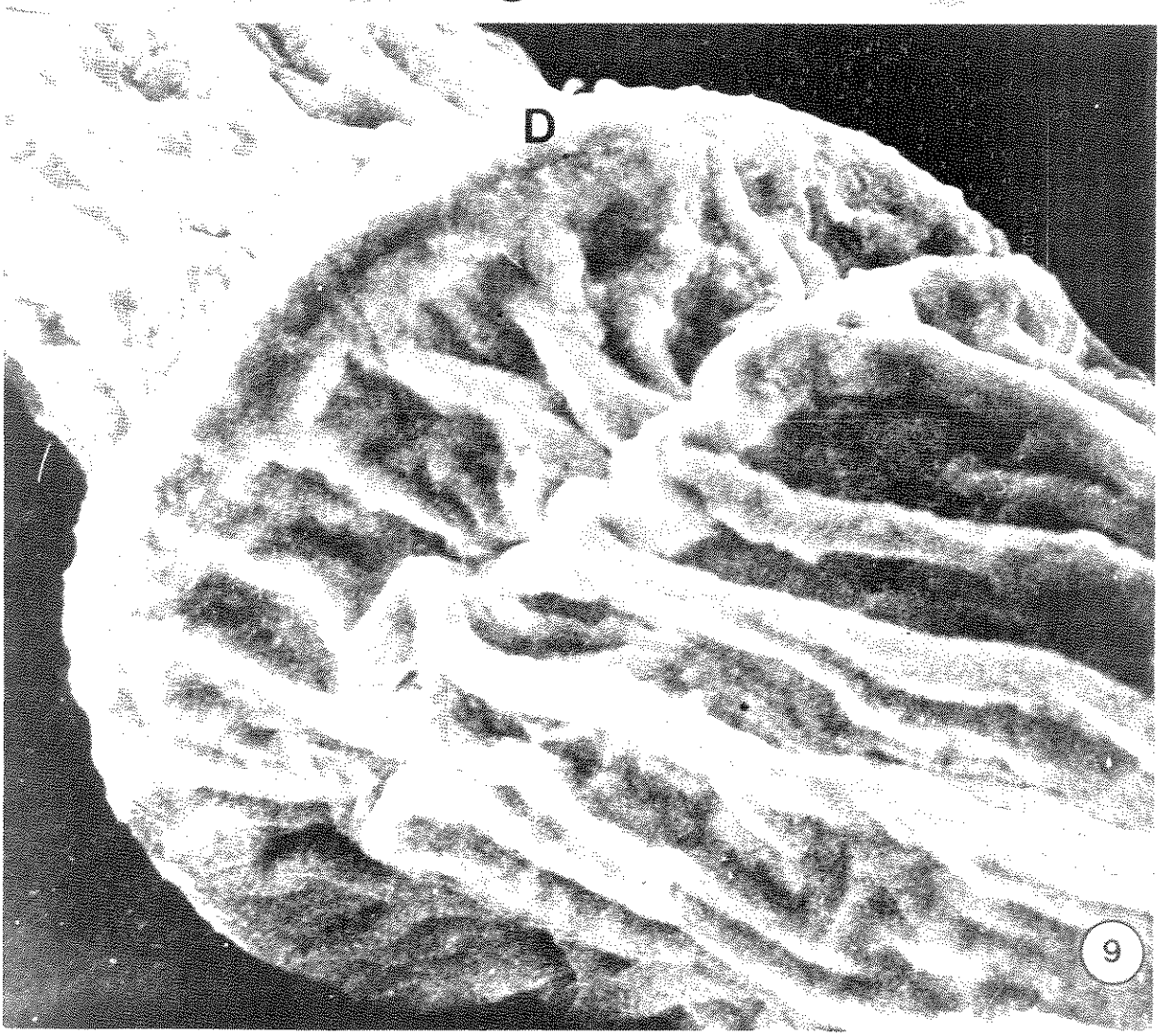
The variation in branching pattern appears to be intimately related to cell wall morphology and chemistry which are extremely important characteristics of the family to which Cladophora and Pithophora belong. It was long believed that the presence of chitin (poly N-acetyl-D-glucosamine; the component of insect exoskeletons) in the cell walls was a distinctive feature of all members of this family. Initial reports of chitin in the outermost wall layers of Cladophora and Pithophora based on histochemical staining (Astbury and Preston, 1940; Tiffany, 1924; Wurdack, 1923), however, were later refuted for Cladophora on the basis of x-ray diffraction analysis and the same histochemical stains (Nicolai and Preston, 1952; Parker, 1964). A more recent biochemical and ultrastructural study of Cladophora indicated that chitin was not present in this alga and that no carbohydrate was detectable in the outermost layers of the cell wall (Hanic and Craigie, 1969). In the light of this finding, the presence of chitin in Pithophora could be legitimately questioned.

Considering that this question is pertinent to both the description of the genus and its relationship to its environment, we conducted a detailed quantitative study to clarify the composition of the cell walls of Pithophora oedogonia and to locate the major structural polysaccharides in the cell walls. The techniques used included cell wall isolation and fractionation, x-ray diffraction analysis, paper and gas chromatography, enzymatic hydrolysis and carbohydrate measurement, amino acid analysis, and light and electron microscopy. These techniques are described in detail by Pearlmutter and Lembi (manuscript in preparation and available on request). In addition, a cytochemical procedure was developed for localizing chitin in the cell wall at the ultrastructural level (Pearlmutter and Lembi, 1978). In this method, the cells are hydrolyzed with potassium hydroxide and the resulting deacetylated polysaccharide (chitosan) can be visualized by treating the tissue with osmic acid. Areas of chitin deposition appear dark brown by light microscopy and electron-dense in the electron microscope.

With standard light microscopic techniques, a double wall is visible around both the akinetes and filamentous cells of P. oedogonia (Fig. 7). The inner wall (IW) encircles individual cells while the thinner, outer wall (OW) ensheathes the whole filament. The entire cell wall thickness can account for one sixth of the cell diameter. Generally, akinetes have thicker walls







Figs. 7-9: Fig. 7. Light micrograph of a cross wall between akinetes ($\bar{A}$). Cells treated with 4% KOH followed by 2% acetic acid to cause separation of the inner (IW) and outer (OW) walls. X 346. Fig. 8. Cross wall disk isolated during cell wall fractionation. X 750. Fig. 9. Scanning electron micrograph of a portion of an air-dried filament. The longitudinal walls have collapsed but the cross wall disk (D) has retained its shape. X 3850.

(6.0 μm average width) than filamentous cells (2.2 μm average width), but the thickness can vary considerably. The inner wall that makes up the crosswall between cells consists of fibrillar layers (Fig. 11) and a disk (Figs. 8, 11) which, when air-dried and subjected to scanning electron microscopy, remains rigid and resists collapse (Fig. 9).

The major structural polysaccharides of P. oedogonia cell walls as determined by x-ray diffraction analysis are chitin and cellulose. N-acetylglucosamine (chitin) and glucose were also detected by gas chromatography (Fig. 10) and, from these data, approximately 6% of the cell wall was estimated to be composed of chitin by weight. Non-nitrogenous hexose (e.g., cellulose) composed approximately 65% of the cell wall dry weight. Other sugars, amino sugars, and sugar derivatives detected included N-acetyl galactosamine (a first record of this compound in algae), galactose, arabinose, fucose, mannose, xylose, and galacturonic acid.

Amino acid analyses indicated the presence of 17 amino acids in the hydrolysates of whole walls and isolated outer wall fractions of P. oedogonia (Table 1). Although some of the protein present in whole walls was lost during purification of the outer wall fraction, all of the amino acids present in the intact walls could be detected. The enrichment of several of the amino acids as well as glucosamine residues in the outer wall suggests that both proteins and chitin are concentrated in this layer.

The distribution of the amino acids in the whole and outer walls of Pithophora is similar to the published accounts of the amino acid composition of Cladophora whole and outer walls, respectively (Table 1). There are large quantities of aspartic and glutamic acids in the walls of both algae, and the trace of methionine in Pithophora is in accord with the absence of this amino acid in Cladophora. However, as previously noted, glucosamine residues have not been detected in isolated Cladophora cell walls.

Not only did we find chitin in the outer wall of P. oedogonia but cytochemical studies indicated its presence in the cross wall disks as well (Figs. 11, 12). Cellulose appeared to be confined to the inner wall only. In addition, we found that a chitin-rich layer (CB) originates from the cross wall area to separate the inner and outer wall of the cell (Figs. 11-13). This layer is rarely continuous from cross wall to cross wall since it tapers to an end before it extends to the middle of the longitudinal wall. In germinating akinetes, this electron-dense layer is absent from the portion

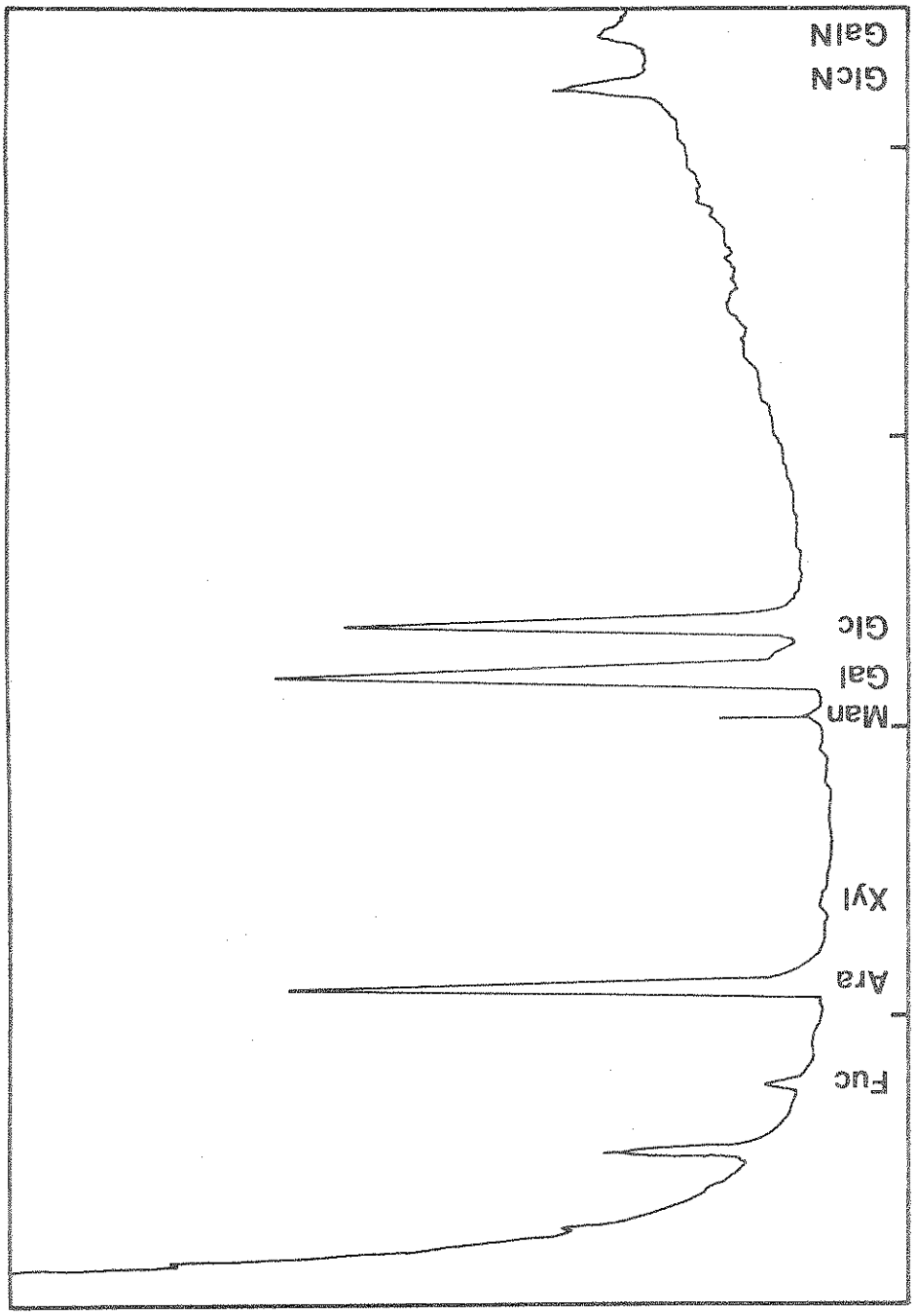
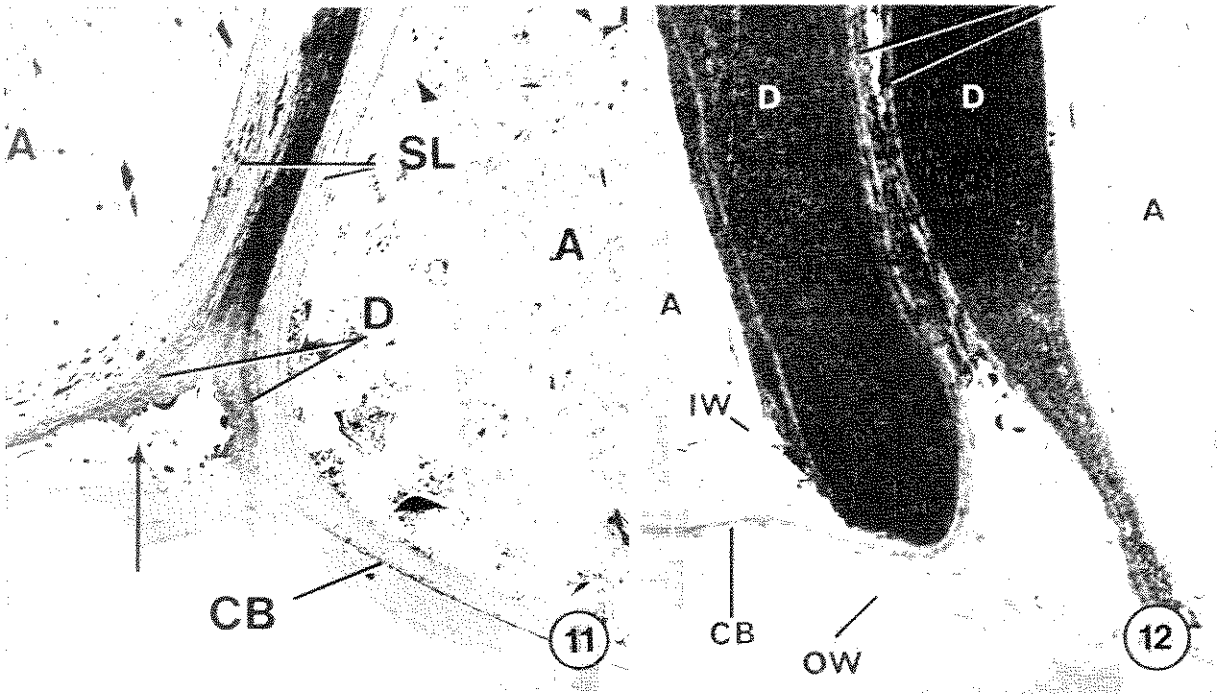


Fig. 10. Gas chromatograph of cell wall carbohydrates from P. oedogonia.

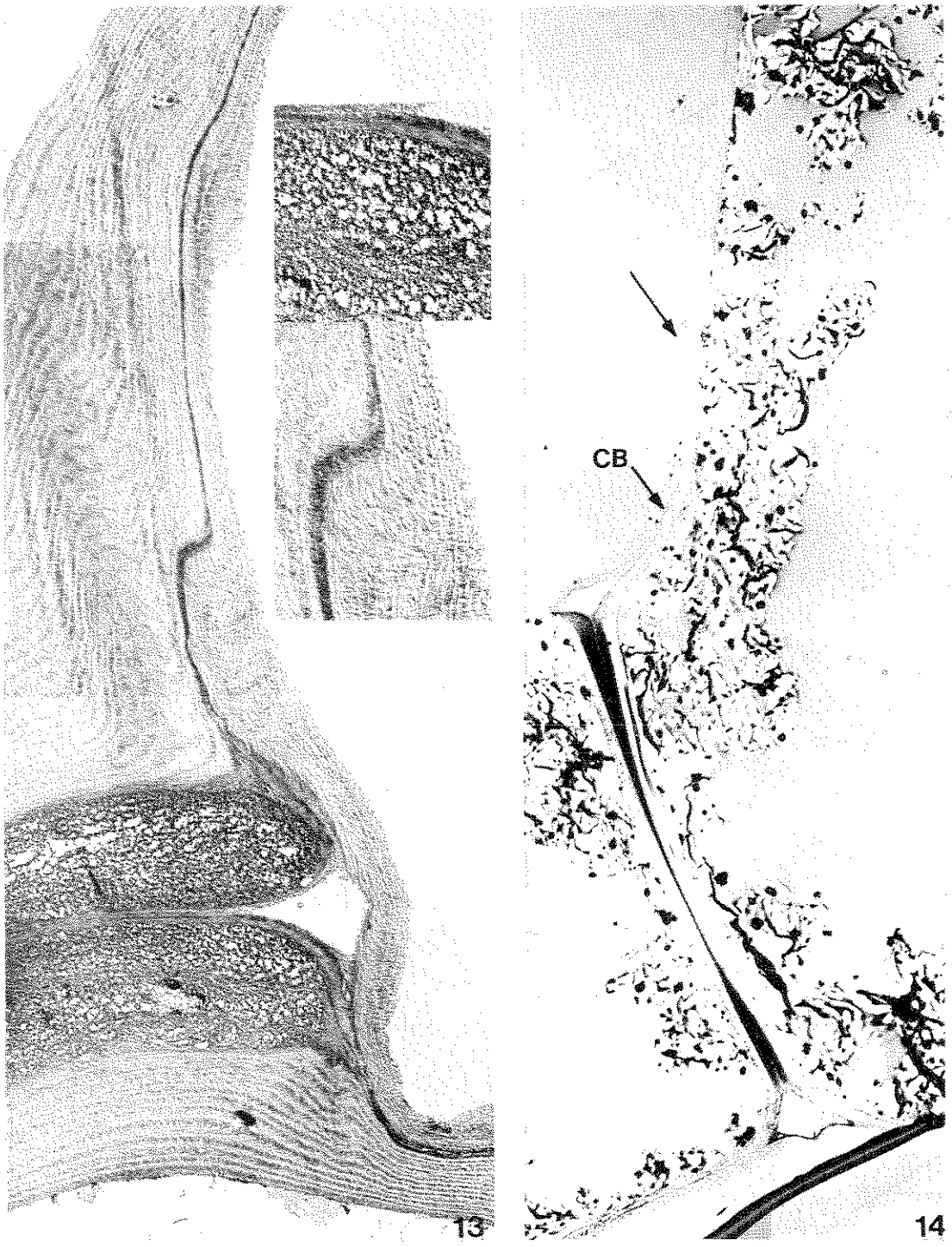
Table 1. Amino-acid and hexosamine composition of Pithophora and Cladophora cell wall fractions

	Whole wall		Isolated outer wall	
	Cladophora[a] (residues/1000)	Pithophora (residues/1000)[b]	Cladophora[c] "cuticle" (+, -)[d]	Pithophora Fraction II residues/1000)[b]
Cysteic acid	64	-	+	-
4-Hydroxy-proline	46	-	-	-
Aspartic acid	130	103	+	75
Threonine	39	73	+ (low)	12
Serine	34	89	+ (low)	15
Glutamic acid	117	109	+	72
Proline	52	68	+	280
Glycine	146	78	+	68
Alanine	65	99	+	49
Valine	55	46	+	60
Cystine (half value)	-	59	-	82
Methionine	-	2	-	2
Isoleucine	26	29	+	18
Leucine	46	38	+	13
Tyrosine	35	37	+	42
Phenylalanine	20	22	+	8
Lysine	71	69	+	84
Histidine	13	9	+	86
Arginine	nd[e]	68	+	32
glucosamine	-	13	-	194
galactosamine	-	7	-	129

[a] Data of Thompson and Preston (1967). [b] Unidentified peaks not included in total.
[c] Data of Hanic and Craigie (1969). [d] (+) Present, (-) Absent. [e] Not determined.



Figs. 11-12: Fig. 11. Electron micrograph of alkali-hydrolyzed and OsO_4-stained cross wall between two akinetes (A). Disk material (D) is electron dense indicating presence of chitosan. The outer wall is separated from the inner wall by a band of chitosan (CB). SL = fibrillar separation layers. X 2900. Fig. 12. Another view of an alkali-hydrolyzed and OsO_4-stained cross wall showing chitosan in the disks (D) and in the layer (CB) separating the inner (IW) and outer walls (OW). X 4100.



Figs. 13-14. Fig. 13. Etched and shadowed cross wall area between two akinetes showing disks and CB layer. Insets are higher magnification of these areas. X 3600. Fig. 14. Longitudinal section through part of a germinating akinete. Akinete was hydrolyzed in alkali and OsO4 stained. The electron dense band of chitosan (CB) originates at the cross wall and tapers to an end at the base of the germination tube (arrow) (this figure best viewed sideways). Portions of the cross wall disks are evident. X 1700.

of the longitudinal wall from which the developing germination tube emerges (Fig. 14).

The site of chitin deposition in Pithophora cell walls (particularly in the cross wall disks and CB layers) provides one explanation for the different branching patterns observed in Cladophora and Pithophora. In addition to published reports, we have looked briefly at several species of freshwater Cladophora (unpublished) and have not been able to detect the presence of chitin. It appears quite likely that Cladophora synthesizes chitin infrequently if at all. Thus, branch initiation in Cladophora occurs at the cross wall probably because of the lack of a chitinous band to restrict cell wall expansion (Fig. 15A). In Pithophora, on the other hand, branch initiation and akinete germination may follow the path of least resistance within the cell wall; i.e., the inner wall expands through that portion of the wall not reinforced by a rigid layer or band (CB) of glycoprotein (Fig. 15B, C). Therefore, branches and germination tubes in Pithophora originate several μm's below rather than directly at the cross wall.

Interestingly, chitin has been confirmed for only a few algae. Chitin was discovered in the chrysophyte Poteriochromonas (Herth et al., 1977) and in pure form (not as a glycoprotein) in the diatoms Thalassiosira fluviatilis (Falk et al., 1966; McLachlan et al., 1965) and Cyclotella cryptica (McLachlan and Craigie, 1966). It is a common component of invertebrate exoskeleton and insect cuticles. In these organisms (and in Pithophora), chitin is never found alone but is covalently bound to protein (Rudall and Kenchington, 1973) to produce a hard, protective layer. (Pure chitin fibers are soft and flexible.)

Like other reinforcing polysaccharides, chitin probably plays a supportive role in P. oedogonia cell walls. The rigid, cross wall disks and the band of chitinous material extending into the longitudinal walls may be the major supportive substance for the very elongate, filamentous cells and may account for the stiffness of Pithophora mats observed in the field. In fact, Pithophora has often been given the common name of "horsehair" alga compared to Cladophora which is called the cotton mat alga (Lopinot, 1971). The lack of chitin in Cladophora cell walls may also account in part for the adaptation of this organism to a flowing water habitat. A dichotomous branching pattern would permit the formation of a thallus providing the least amount of resistance to water flow. Conversely,

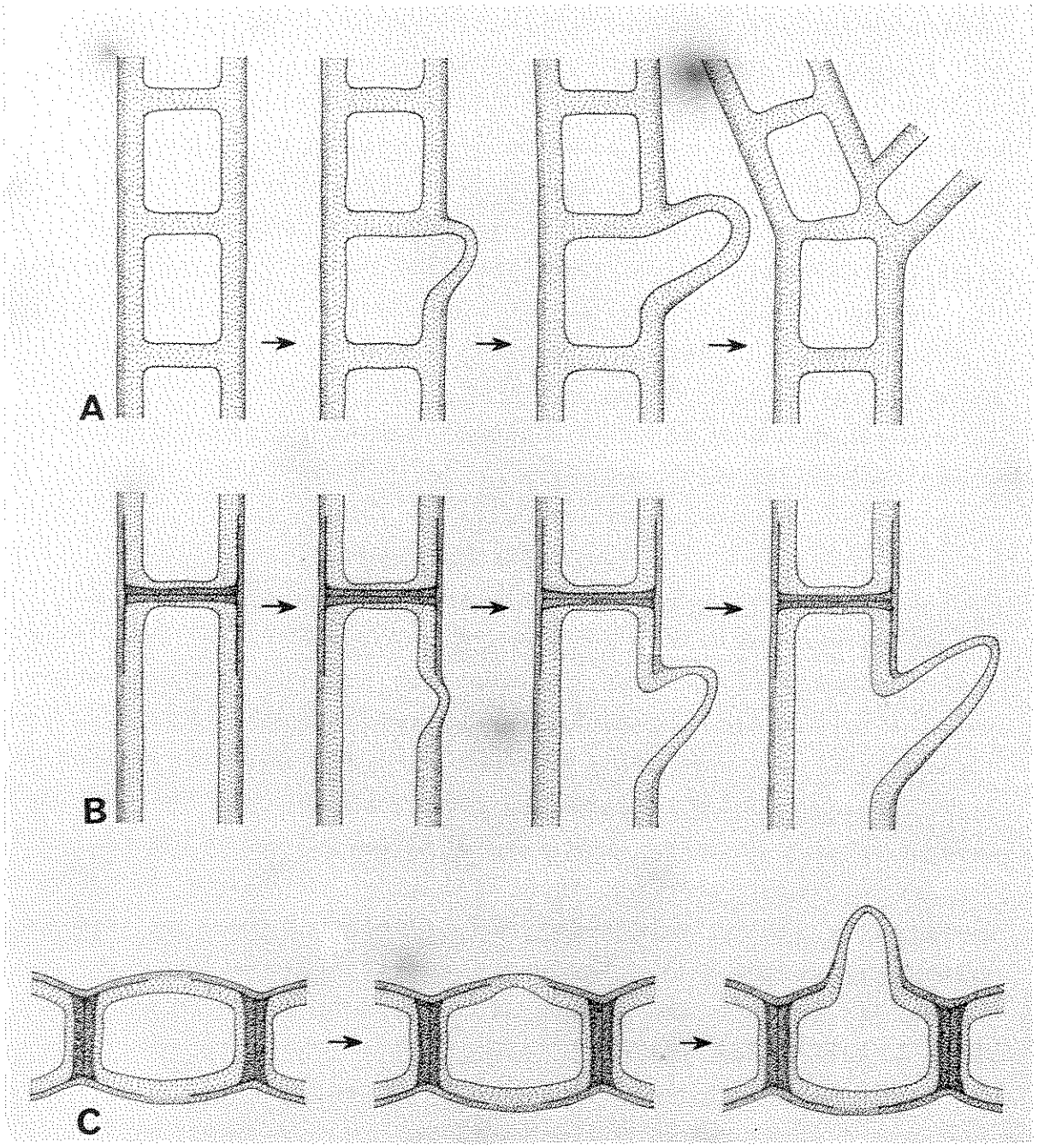


Fig. 15. Diagram comparing branching patterns of Cladophora filaments (A), Pithophora filaments (B), and Pithophora akinetes (C). The heavily stipled areas in B and C indicate sites of chitin deposition.

in stagnant waters such as those inhabited by Pithophora, the stiffness of the thallus and the branching at wider angles may be advantageous by preventing branches from growing in close proximity and thus competing with one another for nutrients and carbon dioxide.

Why does Pithophora synthesize chitin? Previous to this study, cell wall hexosamines were thought to be restricted to heterotrophic organisms whose supply of nitrogen is not as limited as autotrophic plants. Although apparently autotrophic, P. oedogonia grows best in medium heavily supplemented with nitrogen (Neal and Herndon, 1968) and requires high nitrogen concentrations at field sites (see page 77). Thus, the incorporation of hexosamines into the cell wall may be a mechanism by which surplus nitrogen is stored. Evidence of a more substantial nature is required, however, before a relationship between external nitrogen concentrations and chitin synthesis can be confirmed.

Geographic Distribution

At the time Pithophora was first described (1877), the alga was recognized as being primarily tropical in distribution. In fact, Wittrock (1877) collected the original specimens from the Tropical Aquarium-Water Lily House at the Kew Gardens near London, England. Fritsch (1907) considered Pithophora to be a prevalently tropical genus well suited to stagnant waters and Cladophora to be a temperate genus requiring cooler, flowing waters in order to obtain sufficient quantities of oxygen and carbon dioxide. All of the species of Pithophora are found in tropical and subtropical habitats (Patel, 1971; van den Hoek, 1959), and at least in Europe, the organism is probably an introduction. Whether the alga is native or introduced to the Western Hemisphere is unclear, but it was reported in the United States as early as 1887 (Wolle, 1887).

Smith (1950) considered both Pithophora and Cladophora to be widely distributed in the United States but Pithophora to be much less frequently encountered than Cladophora. As might be expected from its world wide distribution, Pithophora is common in farm ponds, lakes and catfish-rearing facilities in the southeastern United States (Weldon et al., 1969). Records of its occurrence in the midwest include those from Michigan and Wisconsin (Prescott, 1962), Illinois (Tiffany and Britton, 1971), and Ohio and Minnesota (van den Hoek, 1959). P. oedogonia, the most frequently encountered species

is also reported for New Jersey, Pennsylvania, and Nebraska (van den Hoek, 1959).

We have found Pithophora to be of widespread distribution in Indiana (Fig. 16). Most of our records are from farm ponds and small lakes which range in surface area from 0.4-8.0 ha. Records from larger lakes (over 40 ha) include Tippecanoe, Wawasee, and Syracuse in Kosciusko Co. and Bel Air in Hillsdale Co., Michigan. In the latter case, approximately 20-30% of this 40 ha lake is infested with Pithophora.

The presence of Pithophora in channelized areas of Lakes Wawasee and Syracuse is particularly important since Wawasee is the largest natural lake in Indiana (1235 ha) and represents a major recreational area in the northeastern part of the state. A survey of the lake in 1971 by a group from Earlham College (Gifford et al., 1971) did not detect the presence of Pithophora although Cladophora mats were noted in the Enchanted Hills section of the lake. The first report of Pithophora by boat owners in the area was in 1973 when heavy growths appeared in the Mud Lake channel at the entrance to Syracuse Lake. The Mud Lake channel is the only connecting link for boat travel between Wawasee and Syracuse, and in that year, a considerable number of boat engines were damaged due to the algae clogging the engine cooling systems. By 1974, the alga had extended its range into Syracuse Lake and throughout the length of the Mud Lake Channel to Wawasee. Continuous copper sulfate treatments were begun in 1974 with little effect. In 1975, the alga was noted in other channelized areas around Wawasee. Portions of the Mud Lake channel were treated with the organic aquatic herbicide Hydrothol 47 (dimethylamine salt of endothall) at approximately 10 times the recommended rate with virtually no results.

In 1976 we conducted an extensive survey of Wawasee, Syracuse, and Papakeechie lakes and found Pithophora to be heavily infesting 8 of the 11 major boat channels in Wawasee (Fig. 17). In addition, approximately 7.0 ha of Syracuse at the Mud Lake entrance were also infested. The alga was both free-floating and layering the bottom of the channels. In every one of these areas, Pithophora was the only macrophyte present. Pithophora was not recorded in any of those channels (Hawaian Isles, Venetian Isles, areas adjacent to the boat channel in Mud Lake) with dense growths of watermilfoil (Myriophyllum spicatum), Chara, and other rooted submersed plants. This exclusion of submersed weeds has also been observed in farm ponds with

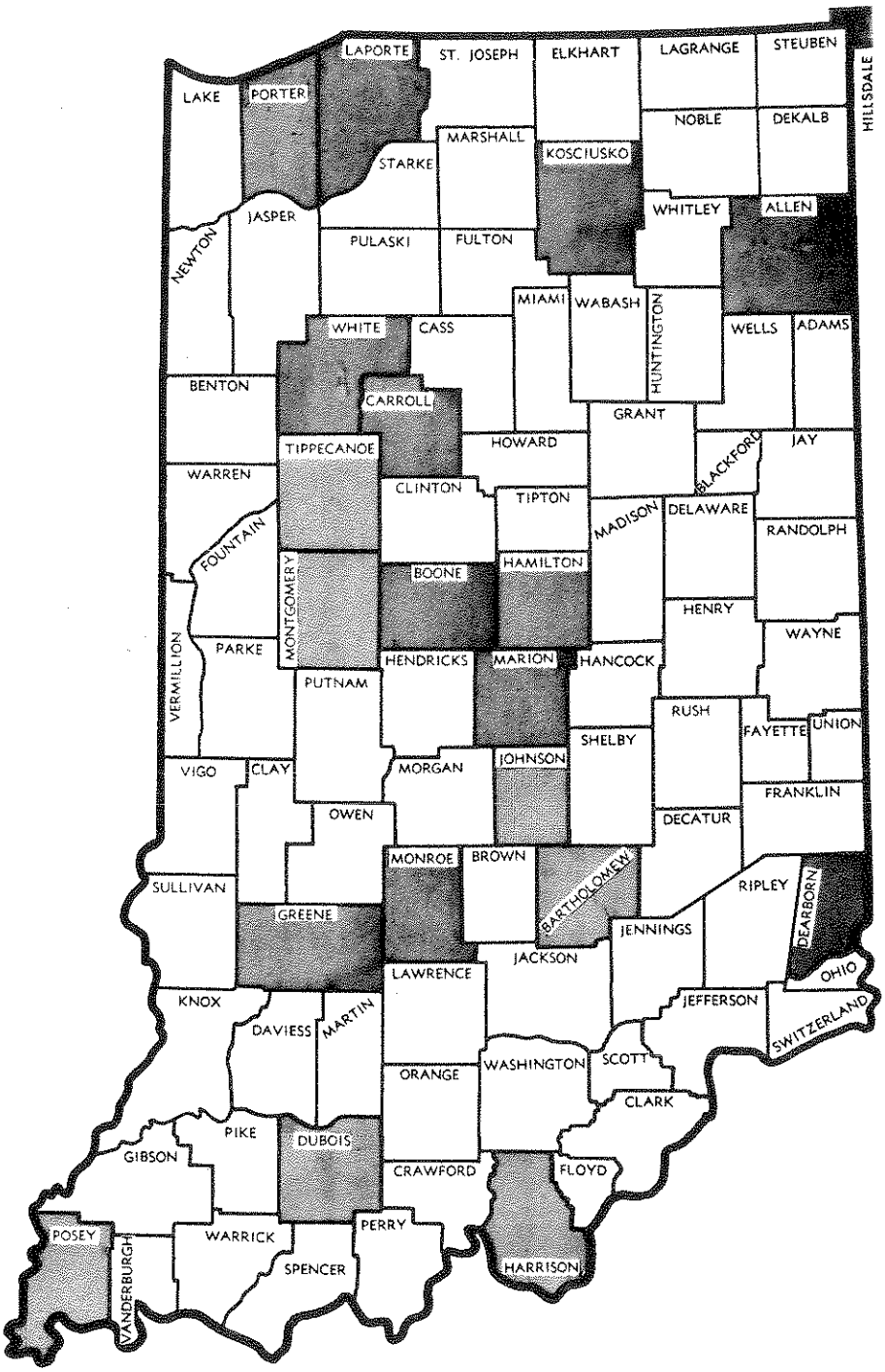


Fig. 16. Indiana counties in which reports of Pithophora infestations have been confirmed as of January 1, 1980. Hillsdale Co., Michigan is also included.

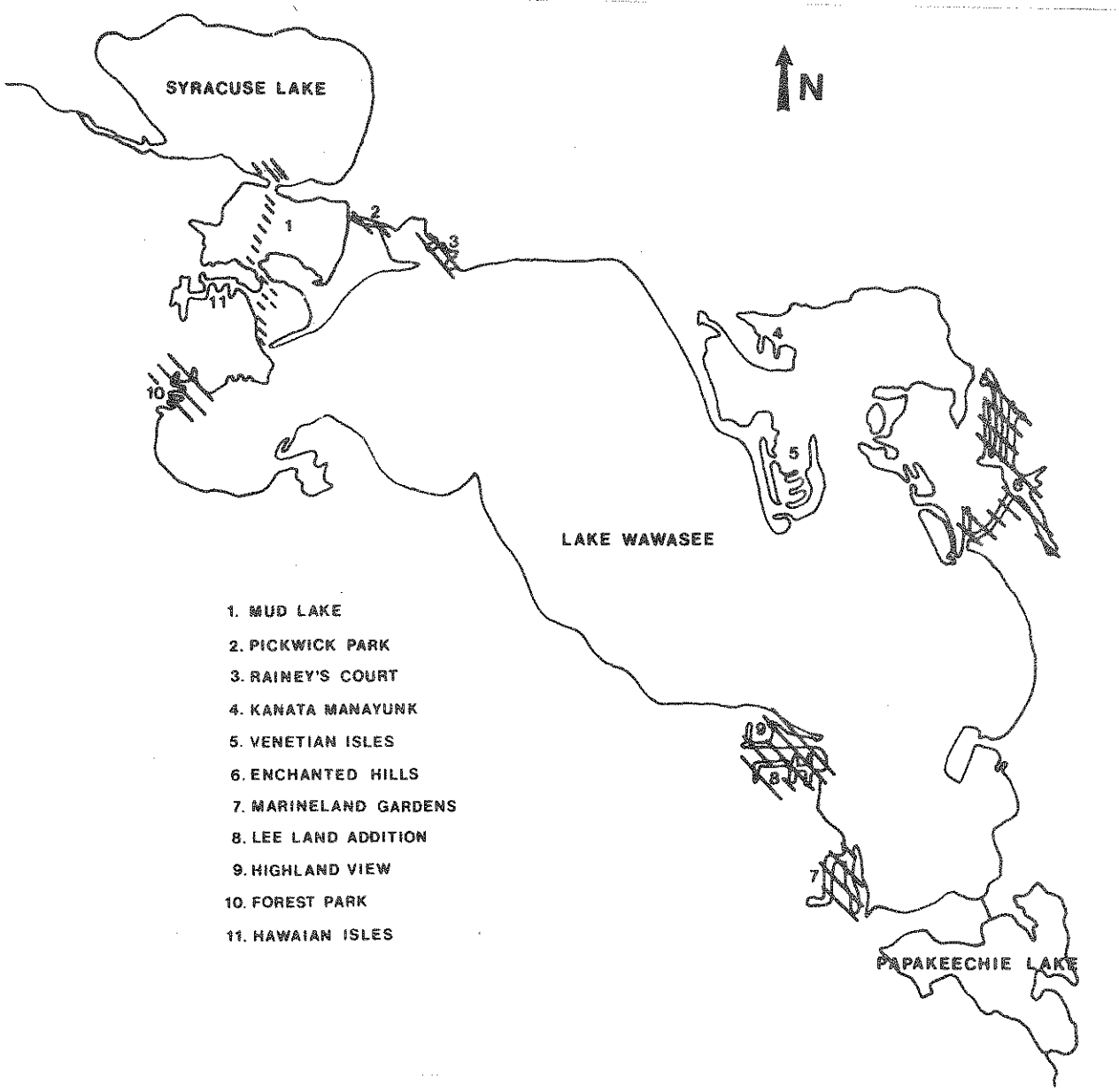


Fig. 17. Distribution of Pithophora (hatch marks) in the channels of the Papkeechie-Wawasee-Syracuse natural lake system. Survey conducted in 1976.

Pithophora infestations. In the Mud Lake channel with its heavy boat traffic, the Pithophora was present in small clumps or balls usually not greater than 10-15 cm in diameter. Biomass measurements taken at monthly intervals at 18 stations in the Mud Lake channel (max. depth = 2.0 m) from June through November ranged from 344.6 g-dry wt m^{-2} on June 21 to 437.2 g-dry wt m^{-2} on August 5 to 348.8 g-dry wt m^{-2} on September 2. These values are higher those that recorded for Cladophora in Lake Ontario (224 g-dry wt m^{-2}) but are comparable to the 280-470 g-dry wt m^{-2} readings reported by Taft (1975) for Lake Erie.

The major species of Pithophora in the U.S. are P. oedogonia and P. varia. There is a great deal of morphological variation among species and consequently much disagreement as to the delineation of species. For example, Collins (1909-18) considered the European species P. kewensis to be identical to P. varia whereas van den Hoek (1959) and other authorities consider P. kewensis to be synonymous with P. oedogonia. Other species of Pithophora include P. aequalis, P. zelleri and P. mooreana.

II. EFFECTS OF COPPER SULFATE ON PITHOPHORA OEDOGONIA

Crance (1974) stated that "few if any herbicides kill Pithophora and give desirable control". The resistance of Pithophora to herbicides such as the most widely used algaecide copper sulfate was recognized as early as 1924 when Tiffany concluded that a concentration of copper sulfate four times greater than that used for other common filamentous green algae such as Spirogyra was required for the control of Pithophora. Eipper (1959) found Pithophora to survive copper sulfate at concentrations 15-20 times greater than the recommended use rate for other algae (0.5-1.0 ppm) while a booklet published by the Phelps-Dodge Corporation on the use of copper sulfate states that Pithophora is highly resistant to the compound and the amount of chemical needed for control has not been established. Chelated versions of copper (e.g., Cutrine Plus) list Cladophora but not Pithophora among species controlled.

One of the obvious mechanisms of resistance in Pithophora is the akinete or spore which supposedly confers protection to the organism from other environmental stresses. Filamentous cells might also show resistance. In either case, the cellular site of resistance is unknown. Several alternatives

are possible. Active compartmentalization of metal ions into nuclei or vacuolar deposits have been reported to account for the resistance of certain metal-tolerant strains of mosses (Skaar, 1973) and algae (Silverberg, 1975; Silverberg et al., 1976 a, b). Some higher plants avoid metal poisoning by the translocation of excess metal ions from the roots to the upper plant tissues (Wu et al., 1975; Wu and Antonorics, 1975). In these plants, the cell wall has been implicated as a non-sensitive site of metal ion accumulation (Turner and Gregory, 1967; Turner and Marshall, 1971). The thick cell walls of fungal spores resistant to copper sulfate have also been suggested as sites of heavy metal ion deposition (Somers, 1963). Tiffany (1924) suggested that the chitinous cell walls of Pithophora may actually block copper sulfate penetration. In these and more recent studies of other metal-resistant organisms, cell wall accumulation of heavy metal ions has been proposed as a means of metal ion exclusion from the more sensitive cytoplasmic sites (Button and Hostetter, 1977).

Using atomic absorption spectrophotometry, biochemical and cytochemical techniques, and electron microscopy, the Pithophora cell type most resistant to copper was determined and the copper ions localized in the cell.

Materials and Methods

Culture. Cultures of P. oedogonia (Montagne) Wittrock were maintained in a modified Bold's Basal medium (Neal and Herndon, 1968) or a 3:1 mixture of modified Bold's Basal medium and soil water at a temperature of 27 C. Light was maintained at 20-65 ft-c with 40 watt, white fluorescent bulbs at a daylength of 12:12.

For several experiments, it was desirable to produce filaments which contained only akinetes or only filamentous cells. This was accomplished by innoculating a few akinete-bearing filament segments into 125 ml flasks filled with 75 ml of medium (approximately 20-40 mg algae/flask). The akinetes usually germinated within 1-3 days and the segments produced filamentous cells exclusively for the first few months. As the nutrients were exhausted, cell elongation ceased and akinetes were formed. After 6-7 months, filaments were composed entirely of akinetes.

Experimental Conditions. Glassware was washed in non-phosphate detergent, rinsed thoroughly in tap water, then distilled water, and finally in glass-distilled water before drying. The alga was exposed to copper in

the light at a temperature of 27 C for 12 h. The temperature and illumination conditions were chosen to simulate applications of copper sulfate to P. oedogonia in ponds where water temperatures are high and available copper is rapidly removed from the water within a day.

Whole cells of P. oedogonia were exposed to solutions of $CuSO_4.5H_2O$ dissolved in incomplete Bold's Basal medium. The phosphate components and the trace element solutions were omitted from this culture medium (hereafter called BBM-1). This allowed the copper treatment to proceed in the presence of most of the ions present in pondwater and necessary for growth, while omitting elments which bind copper or otherwise interfere with copper uptake. The pH of BBM-1 is 6.5 which is close to the pH 6.8 of the modified Bold's Basal medium used to grow and maintain cultures of P. oedogonia. The amount of copper sulfate dissolved in BBM-1 was calculated to obtain concentrations of actual copper in µg/ml (ppm). Field applications of this chemical are usually determined as ppm $CuSO_4.5H_2O$, which is approximately four times the actual amount of copper ion in the final treatment solution.

Isolated cell walls of P. oedogonia were exposed to copper ions in water. The treatment solutions were prepared by diluting copper atomic absorption standard solution (Fisher Scientific Co., Fairlawn, N.J.) in glass-distilled water.

Preparation of P. oedogonia for Copper Toxicity Studies. Filamentous mats were rinsed in BBM-1, cut into 0.5-1.0 cm segments and rinsed in BBM-1 twice. Segments were then divided into small batches weighing approximately 1 mg when dry. Several batches from each experiment were dried to a constant weight to determine the average dry weight per batch of algae. Individual batches of the remaining algal material were then placed in 5 ml of treatment solution in 18 mm glass tubes which were lightly-capped for the duration of the experiment.

Following exposure to copper, the treatment solutions were decanted into clean tubes, and the algal segments rinsed once with distilled water before 5 ml of Bold's Basal medium was added to each tube. The cells were allowed to grow for several days before the number of surviving cells was recorded. For akinetes, survival was measured as the number of spores that had germinated after 3-4 days in copper-free medium. Filamentous cells were maintained in copper-free medium for 2-3 days before the number of unbleached cells was counted.

To determine the amount of copper taken up by dead cells, some akinete-bearing filaments were immersed in 60% ethanol for 45 min and rinsed with water prior to copper treatment. None of the cells survive the alcohol treatment.

Isolation of whole cell walls was attempted using a variety of techniques. Although good breakage of filamentous cells was achieved with some procedures (Braun homogenizer, Virtis homogenizer), only one method successfully opened the thickwalled akinetes. Filamentous mats (about 7 g wet wt) were frozen and mechanically sectioned, either by hand or with a freezing microtome (10 μm sections). The sectioned material was sonicated in ice-cold, glass-distilled water for approximately 20-30 min, filtered on 1-2 thicknesses of nylon mesh (Hanes Hosiery, Inc., Winston-Salem, N.C) stretched over a funnel, and rinsed on the nylon filter with more glass-distilled water. The walls were refrozen and this procedure repeated until microscopic examination revealed that approximately 90-95% of the walls were cleared of cytoplasmic debris. The walls were then gently centrifuged until all suspended material was at the bottom of the tube. This caused the heavier material (walls with cytoplasm still attached) to accumulate beneath the clean walls which formed a white layer on the top of the tube. Purified walls were collected off the top of the tube and used for composition studies. The remaining walls were refrozen and further purified by repeating the isolation procedure. Purified walls that were not used immediately were refrozen or refrigerated in distilled water with a small amount of toluene added to retard bacterial growth.

For treatment with copper, equal aliquots of walls were pipetted into 12 ml centrifuge tubes. The water was removed following centrifugation, and 5 ml of treatment solution was added to each tube before the tubes were capped. After exposure to copper, the walls were centrifuged and the copper solution decanted into 18 mm glass tubes. The walls were dried and weighed.

Determination of Copper. The copper remaining in all of the tubes was determined by atomic absorption spectrophotometry on a varian Techtron Model AA-6 atomic absorption spectrophotometer. Release of calcium, magnesium and zinc in the decanted solutions from the isolated cell wall experiment was also read by spectrophotometry. In all experiments, tubes containing only 5 ml of the treatment solutions were held under the same conditions as tubes which contained algae. To determine the amount of copper absorbed by the glass,

the solutions in these tubes were poured into clean 18 mm tubes and the copper remaining in solution was measured. The amount of copper taken up by P. oedogonia cells or cell walls was then determined after corrections were made for the amount of copper adsorbed by the glass.

In one experiment, where the cellular distribution of copper was to be determined, 15 tubes each of copper-treated and untreated akinetes were harvested, rinsed briefly in distilled water and hand-sectioned with a razor blade in a minimal amount of cold, 0.1 M sucrose. After the cell walls were separated from the cytoplasm by centrifugation, the cytoplasm was centrifuged at 100,000g for 1 h to separate four fractions; a lipid layer, a supernatant, and a two-layered pellet consisting of an upper membrane and a lower starch layer. The cell wall fraction and cytoplasmic fractions were digested with 3 ml concentrated HNO_3, diluted with 7 ml glass-distilled water, and the amount of copper in each of the subcellular fractions was determined by atomic absorption spectrophotometry.

All experiments were repeated at least once except the cell fractionation which, due to a limited amount of culture material, was not replicated. For all other experiments, the data for each copper treatment represents the average of three replications.

Microscopy. For light microscopy, copper was detected with hematoxlin. After a brief rinse in distilled water, cells were immersed in a 0.5 percent aqueous solution of hematoxylin for 3-5 min. Copper binds hematoxylin and the cells were then rinsed in tap water to remove the unmordanted dye, and also to produce a blue stain (due to hematoxylin in alkaline tap water).

For electron microscopy, copper was detected by modifying the silver-sulfide method of Timm (Brunk et al., 1968; Timm, 1960). Untreated and copper-treated cells of P. oedogonia were rinsed briefly in distilled water and fixed in 1% glutaraldehyde in 0.05 M potassium phosphate buffer (pH 6.9) for 1 h. The cells were rinsed twice in buffer and twice in water. To convert the copper to CuS, H_2S gas was bubbled for 10-15 min through the water in which the cells were immersed, and the cells were rinsed in three 15 min changes of distilled water.

This reaction is not specific for copper since several metal ions can be converted to their sulfides by H_2S. Thus, NaCN, which selectively removes CuS, was used as a control to be sure that only CuS was being detected (Goldfischer, 1967; Timm, 1961). A portion of the cells were

immersed in 0.5 M NaCN for 5 min and then rinsed with 3 changes of distilled water for 30 min.

To visualize the CuS, the cells were immersed for 45 min in the light in a developing solution which contained a silver salt ($AgNO_3$) and a reducing agent (hydroquinone). By this procedure, the reduction of silver is catalyzed by the CuS and an electron-dense shell of molecular silver accumulates on the CuS molecule. The developing solution was prepared fresh by mixing 0.1 ml of $AgNO_3$, 8 ml of 10% gum arabic and 2 ml of a mixture of 2 g hydroquinone and 5 g citric acid in 100 ml of water. Some cells were immersed in the developing solution with the $AgNO_3$ omitted as a control for extraneous silver.

Following silver reduction, the cells were rinsed for 30 min in 3 changes of water and embedded in warm, 2% agar. Cells were postfixed in 1% OsO_4 in potassium phosphate buffer overnight at 4 C and rinsed with 3 changes of water for 45 min before they were dehydrated in acetone and embedded in Luft's EPON (Luft, 1961). All procedures except postfixation were carried out at room temperature.

To observe the loss of cellular copper over a period of time, some copper-treated filament fragments were rinsed, placed in copper-free medium, and prepared for electron microscopy (as described above) 0, 2, 4, 16, and 32 h after copper treatment. All materials were sectioned and viewed in the electron microscope with no further staining.

Results

Response of Akinetes and Filamentous Cells to Copper. When akinetes and filamentous cells were treated with copper for 12 h, more akinetes than filamentous cells survived the exposure (Fig. 18). The difference between the spores and vegetative cells was greatest at copper concentrations ranging from 1-4 µg/ml copper. At lower concentrations, viability of both types was high. Most cells could not withstand the higher copper levels, but 1-2% of the akinetes often survived an 8 µg/ml copper (32 ppm copper sulfate pentahydrate) exposure. When these akinetes were examined, they were usually on a single filament fragment. In fact, most of the cells on a segment tended to react similarly upon exposure to copper, and, rather than a random distribution of viable and non-viable cells, many of the surviving cells would be restricted to a few fragments, with the dead cells confined to other fragments.

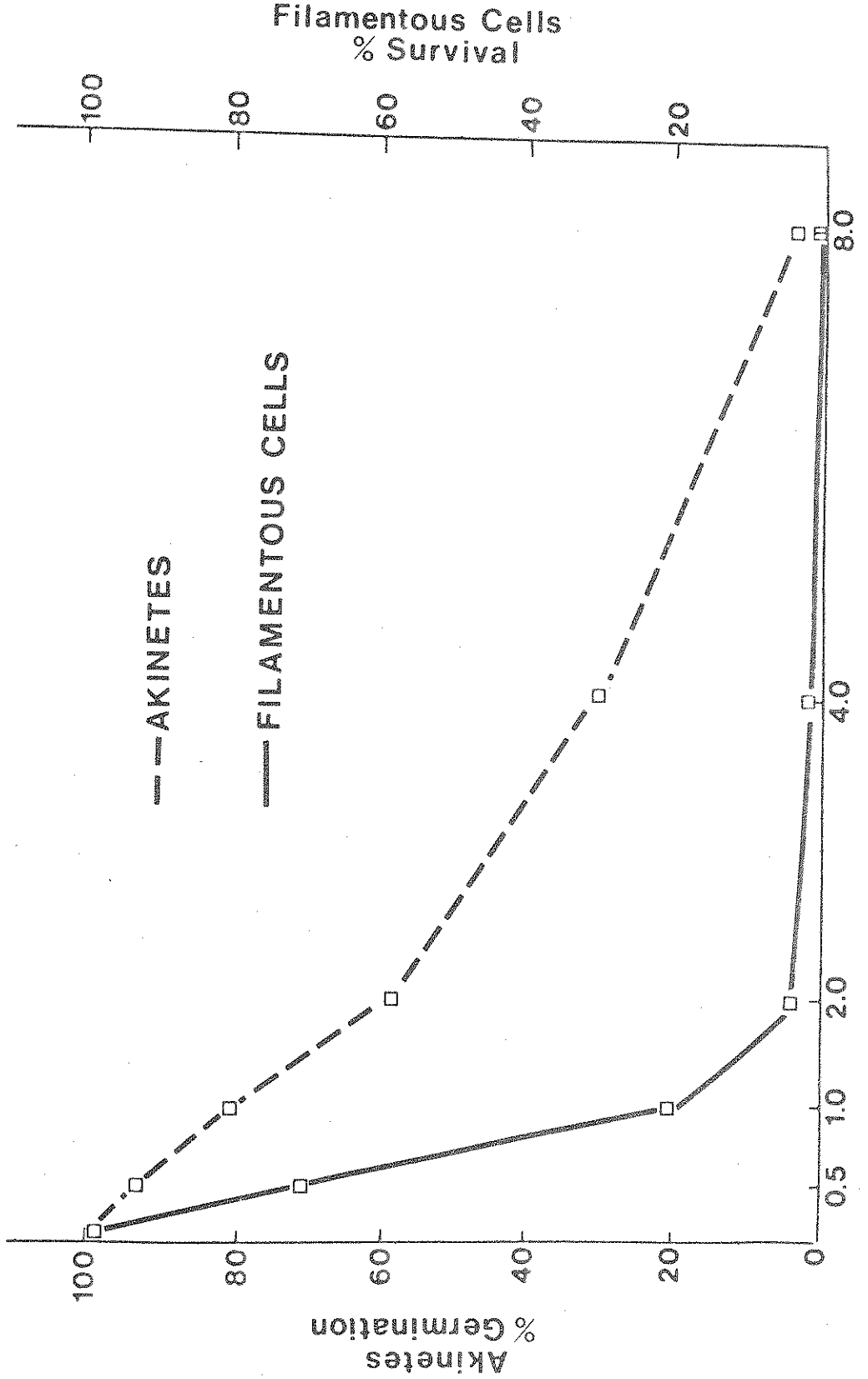


Fig. 18. Viability of akinetes and filamentous cells following exposure to copper.

The highest exposure tolerated by filamentous cells (3% survival) was 2 µg/ml copper or 8 ppm copper sulfate.

Cellular Distribution of Copper. The distribution of copper in fractionated akinetes is shown in Table 2. Untreated cells contain very little copper but a small amount was detectable in the cell wall and supernatant. Almost half of the copper in the copper-treated spores remained in the cell wall fraction. Very little copper was retained by the starch and lipid fraction and even this small amount may be due to contamination from other fractions. Most of the cytoplasmic copper was detected in the supernatant (composed of soluble proteins and ribosomes) rather than bound to membranes (high speed pellet).

Since a treatment of 2 µg/ml Cu^{++} routinely causes mortality to 40% of the akinetes treated, a second experiment was conducted to determine if copper accumulation differs between dead and viable cells. As shown in Table 3, dead akinetes accumulate approximately 1.5 times as much copper as viable akinetes.

Uptake of Copper by Isolated Cell Walls. The two replicate experiments involving copper uptake and divalent cation release from P. oedogonia cell walls are shown in Table 4. Although the response to increased copper concentration was similar in experiments 1 and 2, the studies are separated because the amount of copper uptake per wall dry weight was significantly different in each study.

The results of experiment 2 are also more variable because, due to difficulty in obtaining the large amounts of cultured material required for this experiment, walls from cells of different ages had to be used. Much of the cell wall material in experiment 2 was isolated from filamentous cells whereas in experiment 1, a higher percentage of akinete cell walls were used.

In both experiments, however, the uptake of copper tended to increase as the copper concentration was increased. In experiment 1, the uptake doubled as the concentration doubled. This same trend was observed in experiment 2, although, at higher copper concentrations, the smaller amount of material was probably nearing saturation.

The millimoles of calcium and magnesium released in both studies also doubled as the copper uptake doubled, except at the highest level of added copper in experiment 2. Here the release of calcium, magnesium and zinc leveled off as the copper uptake decreased. Overall, the total number of

Table 2. Distribution of copper in subcellular fractions of P. oedogonia.

	Control	Treated (2 µg/ml Cu^{++})	
	Cu^{++}	Cu^{++}	
	µg/ml	µg/ml	% Total
Cell Walls	0.04	0.91	44.1
Lipid	0.00	0.08	4.0
Starch	0.00	0.03	1.7
High Speed Pellet (Membranes)	0.00	0.25	12.0
100,000 g Supernatant	0.01	0.78	38.2

Table 3. Cu^{++} uptake by live and dead akinetes.

	Cu^{++} Uptake (µg/ml/mg)		
Cu^{++} Treatment (µg/ml)	Live Akinetes	Dead Akinetes	Dead/Live Ratio
0	0	0	0
1.0	0.385	0.645	1.68
2.0	0.550	0.785	1.43

Table 4. Cu^{++} uptake and Ca^{++}, Mg^{++}, and Zn^{++} release by isolated cell walls of P. oedogonia.

Experiment 1: Average Dry Weight Cell Walls/Sample = 8.0 mg.

Cu^{++} Treatment ($\mu g/ml$)	Uptake (mM)	Release (mM)			
		$Ca^{++}+Mg^{++}+Zn^{++}$			
	Cu^{++}	Total	Ca^{++}	Mg^{++}	Zn^{++}
0	0	0	0	0	0
2.0	1.97	2.00	0.75	1.23	0.02
4.0	4.72	3.99	1.50	2.47	0.02
8.0	10.23	8.20	3.24	4.94	0.02
16.0	16.21	15.73	6.24	9.46	0.03

Experiment 2: Average Dry Weight Cell Walls/Sample = 2.6 mg.

Cu^{++} Treatment ($\mu g/ml$)	Uptake (mM)	Release (mM)			
		$Ca^{++}+Mg^{++}+Zn^{++}$			
	Cu^{++}	Total	Ca^{++}	Mg^{++}	Zn^{++}
0	0	0	0	0	0
2.0	9.13	8.16	3.74	4.11	0.31
4.0	13.94	16.15	8.23	7.49	0.43
8.0	26.75	26.82	11.73	14.23	0.86
16.0	38.71	34.34	16.07	17.32	0.95

millimoles of calcium, magnesium and zinc released into solution by the cell walls equaled the millimolar concentration of copper sorbed by the walls at any given treatment concentration.

Microscopy. Akinetes that remained viable following exposure to 2 µg/ml copper germinated more slowly than untreated akinetes (Fig. 19). Two to three days after transfer to copper-free medium, germination tubes were barely visible on copper-treated akinetes whereas the germination tubes on the untreated cells had extended 1-2 mm. Electron microscope observations of the cytoplasm in untreated and viable copper-treated akinetes revealed few differences between the cells, however. Chloroplasts appearing to have a retarded development and a few degenerated nuclei were noticed in the copper-treated akinetes but, after 2 additional days growth, even these differences were not observed. No deposits of copper in specific organelles were found.

Using hematoxylin, copper uptake by cell walls was demonstrated in the light microscope. When copper-treated akinetes were allowed to germinate in copper-free medium, the old cell wall retained the hematoxylin, indicating the presence of copper (Fig. 20). The wall of the germination tube produced in copper-free medium also gave a positive reaction for copper. Neither the old akinete wall nor the germination tube wall of untreated akinetes retained the hematoxylin (Fig. 21).

The effects of copper treatment were also noticed after several weeks growth in copper-free medium. The morphology of the newly-formed filamentous cells was unusual. Instead of the elongate, straight cells that were formed on controls (Fig. 22), cells formed by copper-treated akinetes were often bulbous and short (Fig. 23). Branches on these cells were initiated early and several germination tubes were often produced by one akinete. Many of the newly-formed filaments (and some of the non-viable akinetes) were attached to the culture tube.

After preparation of the cells for electron microscopy, viability and color (gray-green or dark green for copper-killed akinetes) could no longer be used as an indication of cell survival. However, copper-killed akinetes were considerably plasmolyzed following fixation and embedding procedures, and plasmolysis was used as a criterion to distinguish non-viable copper-treated spores.

In the electron microscope, silver deposits indicated the location of

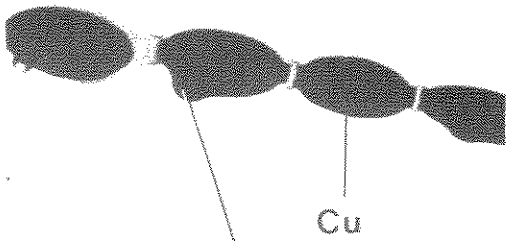
Fig. 19. Copper-treated (Cu) and non-treated (Co) akinetes 2-3 days after transfer to copper-free medium. Glutaraldehyde-fixed and OsO_4 stained. GT = germination tube. X 90.

Fig. 20. Copper-treated akinetes stained with hematoxylin. Both akinetes and germination tubes stained although tubes produced later after transfer to copper-free medium. To observe cell wall stain, akinetes were broken to remove cytoplasm. X 57.

Fig. 21. Untreated akinetes stained with hematoxylin. Compare with Fig. 20. Cell walls are unstained. X 53.

Fig. 22. One to two-week-old untreated germinating akinetes. Germination tubes (GT) have elongated into straight, cylindrical cells. No branches have been produced. X 57.

Fig. 23. One to two-week-old copper-treated akinetes. Germination tubes (GT) are bulbous and dichotomously branched (B). X 59.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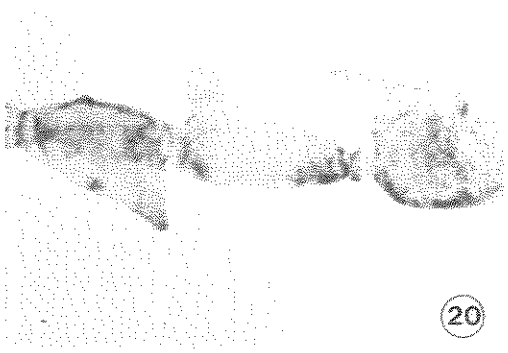
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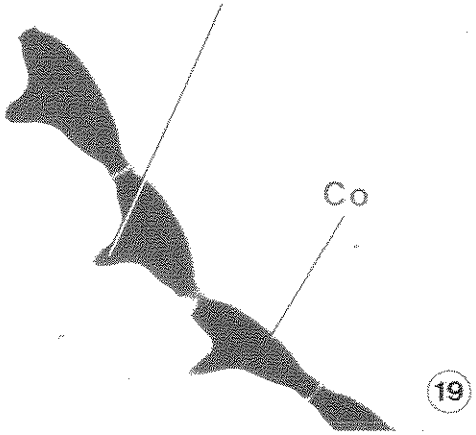
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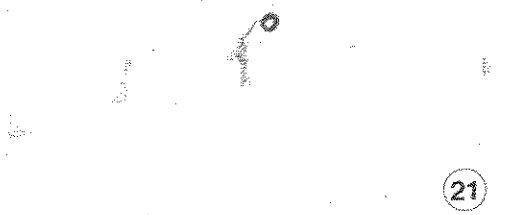
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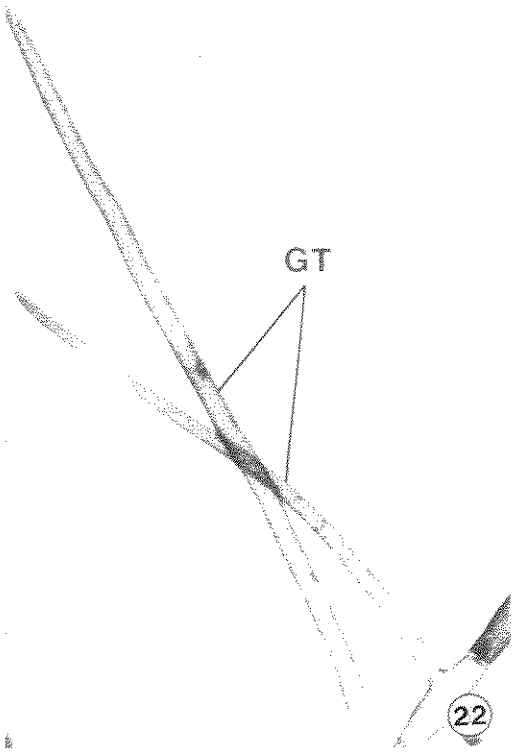
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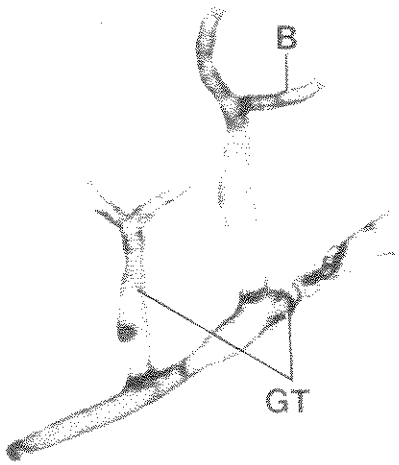
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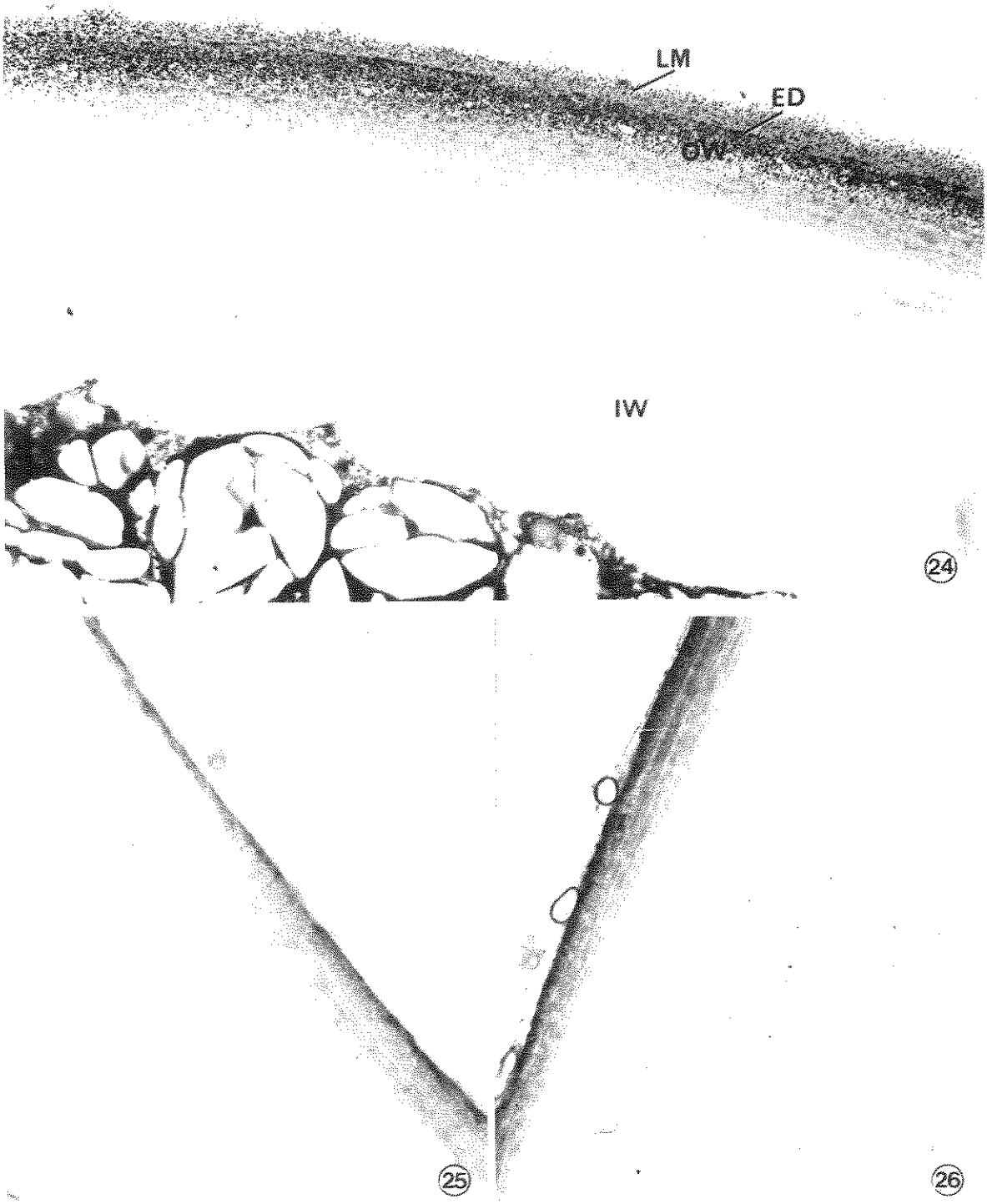


Figs. 24-26: Fig. 24. Longitudinal wall of a copper-treated akinete stained by the silver-sulfide method. Silver present in the outer wall (OW and ED) and cell surface (LM). No silver is present in the inner wall (IW). X 10,000. Fig. 25. Longitudinal akinete wall treated with KCN. Copper from treatment was removed with the KCN prior to silver reduction. No silver is present. X 11,538. Fig. 26. Longitudinal akinete wall treated as a control for extraneous silver. Akinete exposed to copper and stained by the silver-sulfide method with $AgNO_3$ omitted from developing solution. X 12,500.

sorbed copper (Fig. 24). No silver was detected in either of the controls (Figs. 25, 26) and very little was observed in the cytoplasm of viable cells. Most of the silver granules were in the cell wall and were concentrated in the outer wall, the electron-dense zone (ED), and along the surface of the cell (LM). No silver was seen in the inner wall even when copper (as silver) had penetrated into the cytoplasm.

When silver was detected in the cytoplasm, it was not associated with specific organelles. In non-viable akinetes, silver was observed randomly distributed throughout the cytoplasmic and vacuolar regions with the exception of starch and lipid deposits, from which it appeared to be excluded (Fig. 27).

When viable cells were allowed to recover in copper-free medium, the silver deposits were reduced in the cell wall. After 4 h, the number of silver granules decreased on the surface of the cell although heavy deposits were still observed in the outer wall (Fig. 28). Sixteen hours later (Fig. 29), most of the silver was removed from the cell surface and the amount of silver in the outer wall was also reduced. After 32 h, a few silver granules were detectable in the outer wall but were completely gone from the cell surface (Fig. 30). Examination of these cells revealed no silver granules in the cytoplasm.

Discussion

Whitton (1970a), in a study of heavy metal toxicity to algae, could not demonstrate metal-tolerance in Cladophora. He suggested that the large doses of $CuSO_4$ required to control the alga in midwestern farmponds was due in part to the large biomass of the alga in the ponds. In another publication (Whitton, 1970b), he suggested that calcium and magnesium ions in hard or saline waters interfered with copper binding, thus reducing toxicity.

P. oedogonia is extremely resistant to applications of $CuSO_4$ in farmponds. Although environmental factors such as those suggested by Whitton may reduce the toxicity of copper to Pithophora in the field, the present study suggests that P. oedogonia should be considered a copper-tolerant alga because akinetes are several times more copper-resistant than filamentous cells (Fig. 18). The copper resistance observed in P. oedogonia akinetes may be due to three major factors:

1.) Cell surface to volume ratios. Akinetes are short and barrel-shaped

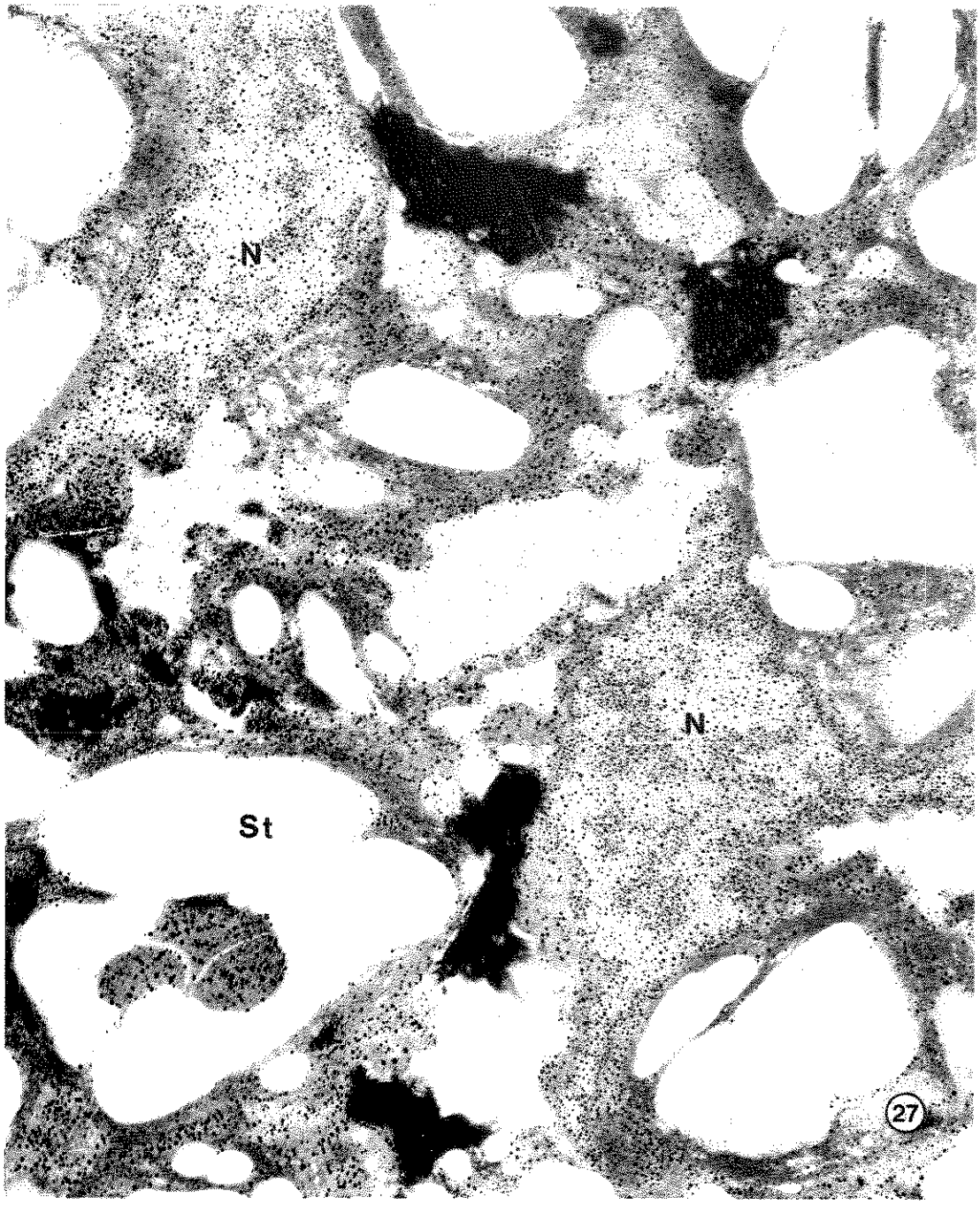
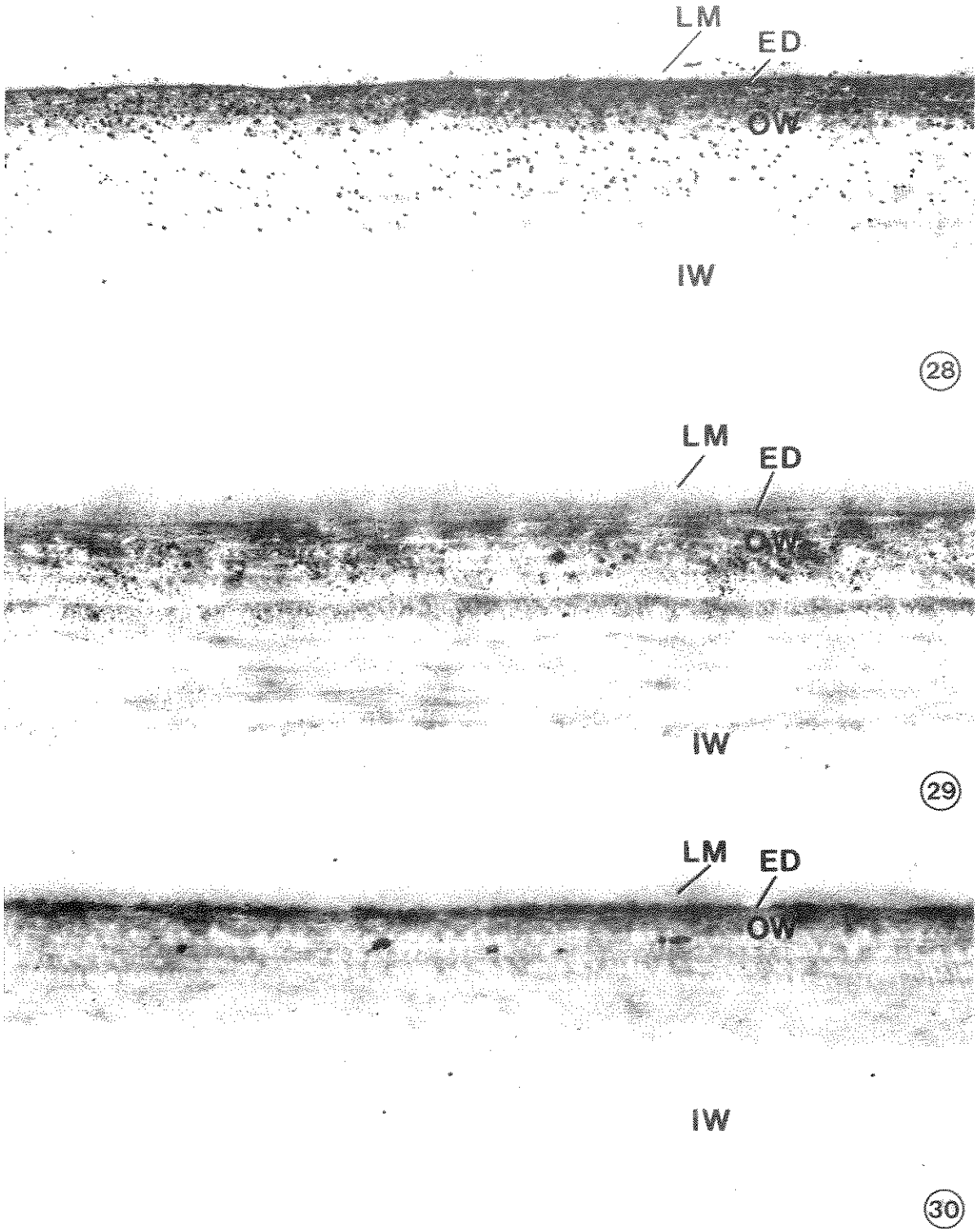


Fig. 27. Cytoplasm of a non-viable akinete stained by the silver-sulfide method. Silver granules are present throughout the cytoplasm except in starch (ST) deposits. N = nucleus. X 22,000.



Figs. 28-30: Fig. 28. Outer wall of a copper-treated akinete after 4 h in copper-free medium. Silver-sulfide stain. Few grains present on cell surface. Compare with Fig. 24. X 10,500. Fig. 29. Outer wall of a copper-treated akinete after 16 h in copper-free medium. Silver-sulfide stain. X 18,000. Fig. 30. Outer wall of a copper-treated akinete after 32 h in copper-free medium. Silver-sulfide stain. Only a few granules remain. X 31,400.

and, when compared to the elongate filamentous cells, have a much smaller surface to volume ratio therefore presenting less surface area for copper uptake. Thus, copper may be less likely to penetrate an akinete than a filamentous cell. In a study of lead uptake in algae, Stokes et al. (1973) noted that Cosmarium, which had a higher surface to volume ratio than the other species tested, was also the most susceptible alga to lead poisoning. Although a large cellular surface area may provide more non-sensitive wall-binding sites, it may also increase the susceptibility to metal poisoning if the cytoplasmic volume is small in comparison.

2.) Metabolic activity. Akinetes tend to be dormant structures, maintaining the cell in a low metabolic state under adverse environmental conditions (depleted nutrients, low temperature or illumination levels). Filamentous cells, on the other hand, are actively growing and transporting substances across the plasma membrane into the cell. Thus, there is a greater probability that copper ions, bound to substances on the cell surface, will be carried across the cell membrane of filamentous cells and eventually released into the cytoplasm.

3.) Cell wall binding. The cell wall and cell surface of the akinete are important sites for the accumulation of copper. In the fractionation study, almost half of the copper taken up by akinetes was found to be in the cell wall fraction. If all of the akinetes used in this study had remained alive during copper treatment, the percentage of copper in the cell wall fraction would probably have been much higher. Since we found that dead cells accumulate more copper than viable cells, it is probable that mass flow of copper into the dead cells accounted for at least a portion of the uptake by the cytoplasmic fraction. Other researchers have noted that once the plasma membrane of a cell is disrupted, ions can pass freely into the cytoplasm (Glooschenko, 1969; Hassall, 1963; Somers, 1963). Also, as shown by the silver sulfide detection method, the copper in copper-killed akinetes was found to be evenly distributed in the cytoplasm (although starch grains do exclude copper). We found no evidence for compartmentalization of copper into vacuolar or intranuclear deposits.

Based on the silver sulfide method, it appears that very little copper enters the cytoplasm of a viable cell that has been exposed to copper. Most of the copper taken up by the cells is associated with the outer cell wall and surface of the cell and is easily removed or redistributed throughout the wall

during growth. A small non-lethal dose of copper penetrating to the cytoplasm probably causes the inhibitory (algistatic) effect which results in a delay in akinete germination in relation to untreated controls.

The identity of the copper binding component in the cell wall is as yet unknown. Tiffany (1924) thought that chitin in the cell wall could prevent copper penetration, and indeed our studies have shown chitin to be localized in the outer cell wall. However, the chemical structure of chitin makes it an unlikely candidate as either an attractant or a barrier for cations. A check of purified chitin for copper sorption produced negative results. It is much more likely that copper would bind to anions on the cell surface with the most probable candidates being either proteins which are concentrated in the outer cell wall or phosphate groups, perhaps in the form of inorganic polyphosphates or phosphates bound to glycoproteins. These compounds have been demonstrated to accumulate on the cell surface and cell walls of both algae and fungi (Brandes and Elston, 1956; Harold, 1962; Van Seveninck and Booij, 1964). In addition, enzyme systems for the hydrolysis of phosphorus compounds, in particular, the alkaline phosphatases, are firmly bound to the surfaces of algal cells and require metallic cofactors. Lin (1977) reported that an intracellular alkaline phosphatase of Cladophora was most active if Mg was present in the substrate mixture. Copper replacement of phosphatase cofactors such as magnesium and possibly calcium or zinc suggests one explanation for the ion-exchange reaction we observed using isolated cell walls since our studies show that Pithophora can also produce alkaline phosphatases (see pg. 86).

Although this explanation may account for part of the cell wall uptake of copper, the irregular, morphological changes observed in later stages of growth could be due to a different ion-exchange site in the cell wall. Hematoxylin staining of germinating akinetes suggests that a portion of the copper sorbed by the wall is redistributed during growth. The abnormalities observed could be due to wall loosening as a result of a copper exchange for calcium bridges between polysaccharide chains. Since the crystal radii of copper (0.96 Å) and calcium (0.99 Å) are very close, the substitution of copper for calcium bridges between the polysaccharide chains is not unlikely.

The differential response to copper treatment observed between akinetes and filamentous cells is probably due to the relative amounts of binding constituents rather than to cell wall thickness, particularly since this

parameter can vary a great deal between cell types. There is no reason to believe that copper does not bind to the outer wall layers of filamentous cells, and this certainly could account for their tolerance to doses of copper sulfate (2-4 ppm) which are higher than those used for the control of most filamentous algae.

In this study, most of the copper attached to the outer wall and surface of the cell in P. oedogonia was rapidly lost after the cells were placed in copper-free medium. The copper was probably released back into the culture medium which contained the chelating agent, EDTA. Indeed, the reversible binding of metals to cell walls has been found to be associated with metal tolerance in other algae (Button and Hostetter, 1977; Silverberg, 1975) and higher plants (Turner and Marshall, 1971). In pond environments following copper sulfate treatment, a similar phenomonon probably takes place. After 12-24 h, the ionizable copper concentration in the water decreases, due to dilution or uptake by sediments which can act as chelating agents themselves (Lewis et al., 1973). Midwestern water is hard, alkaline, and contains high concentrations of calcium and magnesium. Although a certain amount of copper probably remains in the akinete cell wall and is redistributed to new cell wall material as viable akinetes germinate, it is likely that most of the cell wall-sorbed copper is released and replaced by calcium and magnesium as the pond concentration of available copper is lowered.

III. LIFE CYCLE OF PITHOPHORA WITH EMPHASIS ON AKINETE PERIODICITY AND TOLERANCE TO STRESS

When we found that akinetes were more resistant to copper sulfate than filamentous cells, we decided that the next step should be to determine the seasonal distribution of akinetes in the field and define the environmental factors regulating their appearance. Although the presence of akinetes in field-collected Pithophora has often been noted (for example, in the early literature: Fritsch, 1907; Mobius, 1895; Mothes, 1930; Tiffany, 1924), there were no reports, to our knowledge, of a regular periodicity in either tropical or temperate populations. In addition, as a prelude to evaluating management techniques (e.g., lake or pond drawdowns) it was necessary to determine akinete viability in water and hydrosoil under extreme as well as "normal" environmental conditions.

All of the field work described in this chapter was conducted at Surrey Lake, a shallow 3.9 ha man-made lake in Bartholomew Co., central Indiana. For a detailed description of the physical and chemical parameters of this lake and the location of the collecting stations, see pages 71-73, Tables 19-20, and Fig.39.

Standard Akinete Germination Test

In the following sections, akinete viability was tested by placing the akinetes, whether collected from the field or from laboratory culture, under a standard set of conditions. Filaments with akinetes were pulled from the vegetation with forceps and, unless otherwise noted, inoculated into C1(II) medium modified by the deletion of the B7 and C13 trace element components (Gerloff and Fitzgerald, 1976) in petri plates. Illumination was provided by a 61 cm cool white fluorescent tube with a 10:14 LD cycle and irradiance of 15 μeinsteins $m^{-2}s^{-1}$. Temperature was 21 C. Akinetes were examined after 7 days and % germination calculated. Akinete numbers ranged from 30 to 100 per plate and a minimum of 4 plates per treatment or collection date was inoculated.

Akinete Periodicity and Viability in Free-Floating Mats

This section deals primarily with the akinete portion of the life cycle of Pithophora. The seasonal distribution and viability of akinetes in free-floating mats are described as are the environmental factors which regulate akinete germination and formation. Although a brief note on seasonal distribution of vegetative biomass is included, more detailed information on this topic is presented in the next chapter, pg. 75.

Seasonal Distribution of Akinetes. Samples of P. oedogonia were collected from Surrey Lake from February, 1978 through June, 1979. The number of akinetes present was estimated in the following manner. The Pithophora mats were washed in tap water to remove sediments and extraneous plants (primarily Lemna). Small clumps of the alga were blotted dry with paper towels and weighed. Ten g of this material were added to 100 ml of water and disrupted in a Waring blender at medium speed for 10 min. The resulting liquid consisted of broken Pithophora filaments and intact akinetes. Akinete counts were made on 1 ml aliquots of the solution dispensed into Sedgewick-Rafter counting chambers (at 40X). The number of akinetes present per gram fresh weight of filaments was then calculated.

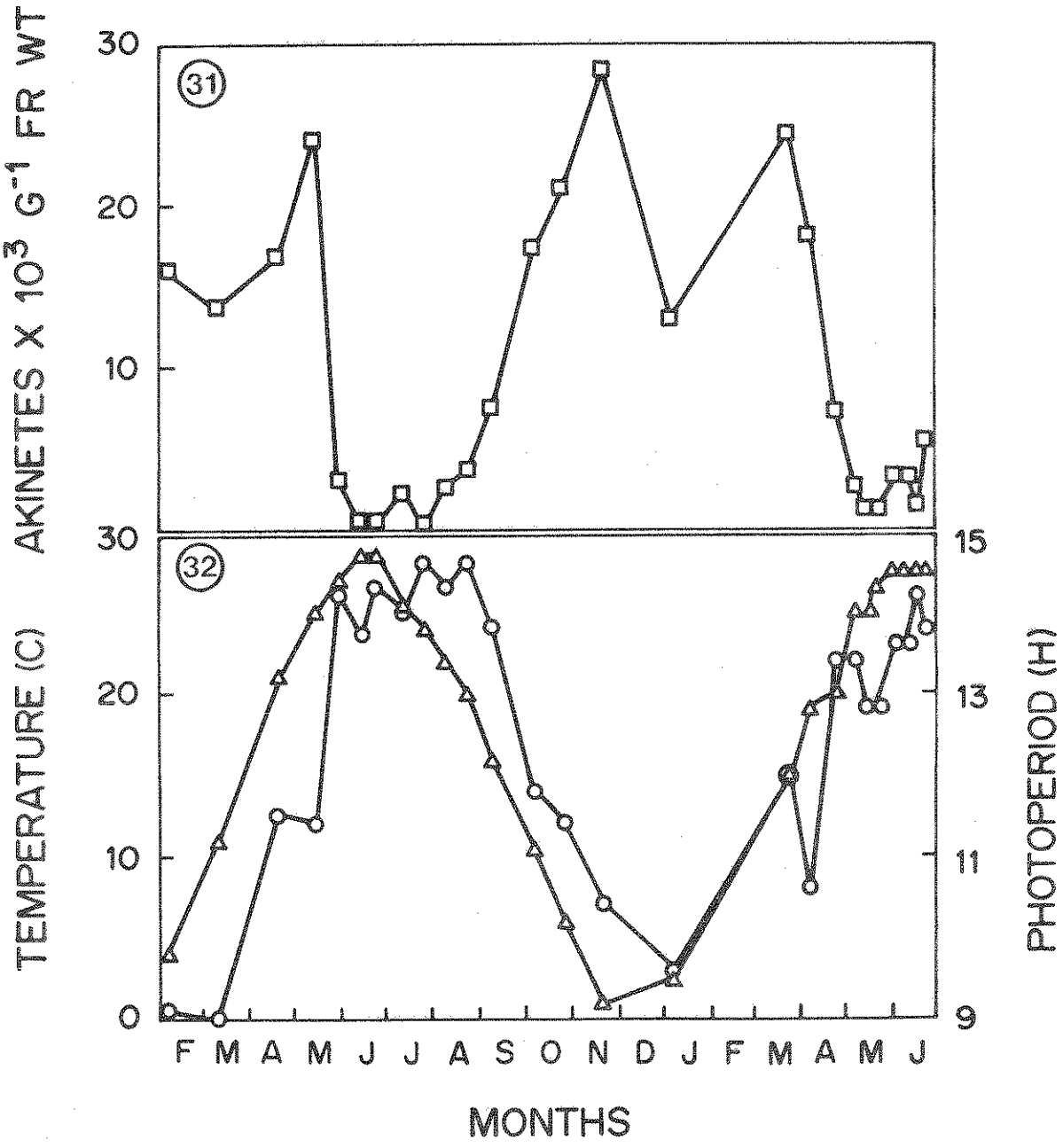
There was a clear pattern of seasonal variation in the abundance of P.

oedogonia akinetes in Surrey Lake with akinete numbers being highest during the winter and early spring, declining to lower levels during the summer, and increasing during the fall (Fig. 31). In 1978, akinete numbers were highest from February through mid-May. A sharp decline in akinete numbers occurred between the May 15 and May 31 sampling dates. Microscopic examination of akinetes collected during this period revealed that germination tubes were present indicating that the observed decline in akinete abundance was due to germination of the akinetes present and subsequent growth of new filaments. Following this period of germination (hereafter termed the germination episode), akinete numbers remained relatively low through August. Numbers for this midsummer period varied from 100 to 2600 akinetes g^{-1}. At no time were akinetes lacking in the vegetation samples. Between late August and mid-October, akinete numbers increased to winter levels. A similar pattern was observed during 1979 except that the germination episode occurred in late April (3-4 weeks earlier than in 1978). Although not shown in Fig. 31, akinete numbers in the fall of 1979 again increased from 3400 akinetes g^{-1} in mid-September to a midwinter value of 20,200 akinetes g^{-1} by October 30.

A similar pattern in periodicity of akinete numbers was observed in 1976-77 in the Mud-Lake Channel between Lakes Wawasee and Syracuse. The counts of akinetes g^{-1} were higher than those from Surrey Lake and were as follows: October, 41,000; December, 67,200; February, 59,850; March 75,875; April, 91,543; and June, 3605.

Seasonal Distribution of Vegetative Biomass. Although we do not have data for 1978 or early 1979, biomass of Pithophora vegetation in Surrey Lake appears to be lowest during the winter (November, 1979) and spring periods prior to (March, 1980) and immediately following (May, 1979) the germination episode (Table 5). The relatively low biomass figures for the summer months are due to the inclusion of data from the deep water stations 8-12 which do not support as great a biomass as the shallow water stations (see Fig. 40).

Environmental Regulation of Akinete Germination. If herbicides such as copper sulfate or copper sulfate combinations are to be used successfully for Pithophora control, the identification of that period in time when akinetes are at their lowest numbers is extremely important. Presumably, rapid akinete germination along with low vegetation biomass (i.e., the germination episode) may be indicative of a period in which the organism will show a high degree of susceptibility to herbicides. Thus, the identification of the major



Figs. 31-32: Fig. 31. Change in akinete abundance in free-floating mats collected from Surrey Lake from February, 1978 - June, 1979. Fig. 32. Changes in water temperature at 0.5 m (o ——) and photoperiod (Δ ——) for Surrey Lake from February, 1978 - June, 1979.

Table 5. Biomass of P. oedogonia vegetation collected from stations 2-12, Surrey Lake. Means calculated on all samples including 0 values. Values given are $X \pm$ S.D.

Date	Biomass Dry wt. ($g\ m^{-2}$)	No. of Samples
May 79	57.4 ± 71.3	11
June 79	103.3 ± 146.5	22
July 79	83.2 ± 87.3	33
August 79	85.3 ± 90.3	55
Sept. 79	121.0 ± 89.7	44
Oct. 79	87.9 ± 117.8	33
Nov. 79	27.1 ± 90.0	11
Mar. 80	69.7 ± 45.1	11

environmental parameter which regulates akinete germination in the field could be of predictive value as an indicator of Pithophora susceptibility.

Relatively little information is available on akinete germination in algae in general (Fogg et al., 1973). In laboratory studies, Neal and Herndon (1968) and Riley and Anderson (1976) reported that P. oedogonia akinetes germinated following transfer to fresh medium. Neal and Herndon also noted that germination occurred in complete darkness as well as light and concluded that light was not required for germination. Conversely, Patel (1971) reported that green light stimulated P. oedogonia akinete germination compared to red, blue, or yellow light. Patel's results, however, may be subject to question as the irradiance was not equal at each of the four wavelengths tested.

By combining the monitoring of akinete levels and environmental conditions in Surrey Lake with laboratory experiments, we were able to determine the factor regulating the germination of P. oedogonia akinetes. Water samples collected concurrently with the algal material were analyzed for NO_3-N by the method of Nelson and Sommers (1975). Soluble-reactive phosphorus was determined on filtered samples (0.45 µm pore size membrane filters) using the ascorbic acid technique (Am. Publ. Health Assoc., 1971). Water temperature at 0.5 m was recorded with YSI Model 33 S-C-T meter. Photoperiod measurements were obtained from the National Weather Service.

Laboratory germination experiments using akinetes collected from Surrey Lake and from laboratory cultures were conducted in C1(II) medium modified by the deletion of the B7 and C13 trace element components (Gerloff and Fitzgerald, 1976), TBIM medium (Smith and Weideman, 1964), and Surrey Lake water. Except where noted, illumination and temperature were provided as described under the standard akinete germination test. Akinetes were examined following 7 days exposure to a particular treatment and % germination calculated.

During the study period of February, 1978 through June, 1979, the photoperiod and water temperature in Surrey Lake changed (Fig. 32) as did akinete numbers (Fig. 31). Photoperiod which increased from 9.5 h in January to 14.7 h in June was inversely related to akinete abundance ($r = -0.53$; $P < 0.001$; $N = 26$). Water temperature ranged from 0.5 C to 26 C. The relationship between water temperature and akinete abundance was also inverse ($r = -0.75$; $P < 0.001$; $N = 26$). Nutrient levels in the lake were generally

higher prior to the germination episode than immediately afterward (Fig. 33).

These observations resulted in three hypotheses concerning environmental regulation of akinete germination: 1) germination occurred in response to increased length of the photoperiod; 2) germination resulted from increased water temperature; and 3) germination occurred in response to high NO_3-N and PO_4-P concentrations in the lake water.

We performed laboratory experiments designed to test each of these hypotheses. In one set of experiments akinetes were incubated under 10 or 13 h photoperiods. These photoperiods were chosen because photoperiods of 9.5-14.0 h (Feb.-May) were observed prior to the 1978 germination episode. The results of these experiments (Table 6) indicated that it was unlikely that short photoperiods were inhibiting akinete germination in Surrey Lake since > 95% of the akinetes exposed to these photoperiods germinated.

The field data indicated a negative relationship between akinete numbers and water temperature and that the sharpest decline in akinete abundance during 1978 corresponded to a water temperature increase of 12.5 to 26 C. To study the effects of temperature on akinete germination we incubated akinetes at 4 temperatures: 10, 12.5, 15, and 20 C. In addition, a 10 h photoperiod was used. The results of this set of experiments (Table 7) clearly showed that germination increased as temperature increased. Akinetes exposed to 10 C had not germinated even after 10 weeks.

It was also evident that NO_3-N and PO_4-P levels were high prior to germination. In a third set of experiments, we examined the effect of NO_3-N and PO_4-P on akinete germination using a two-way analysis of variance design. The treatments for this experiment were 0, 70, 300, and 1170 μM NO_3-N and 0, 23, 45, and 90 μM PO_4-P, resulting in 16 discrete combinations of NO_3-N and PO_4-P with 4 replicates at each. The experiment was conducted using Cl(II) medium at 20 C with a 10:14 LD cycle. Akinete germination was clearly influenced by the level of NO_3-N and PO_4-P present in the external medium (Fig. 34). Statistical analysis revealed that NO_3-N and PO_4-P interacted in a significant manner (Table 8). Further analysis using the Test for Simple Main Effects (Kirk, 1969) elucidated the nature of the interaction. Increased NO_3-N levels led to greater akinete germination at each PO_4-P level tested (Table 8). PO_4-P, however, had a significant effect only at the intermediate levels of NO_3-N tested. Of greater relevance, however, is the fact that good germination occurred in medium containing 70 μM NO_3-N at all 4 levels of

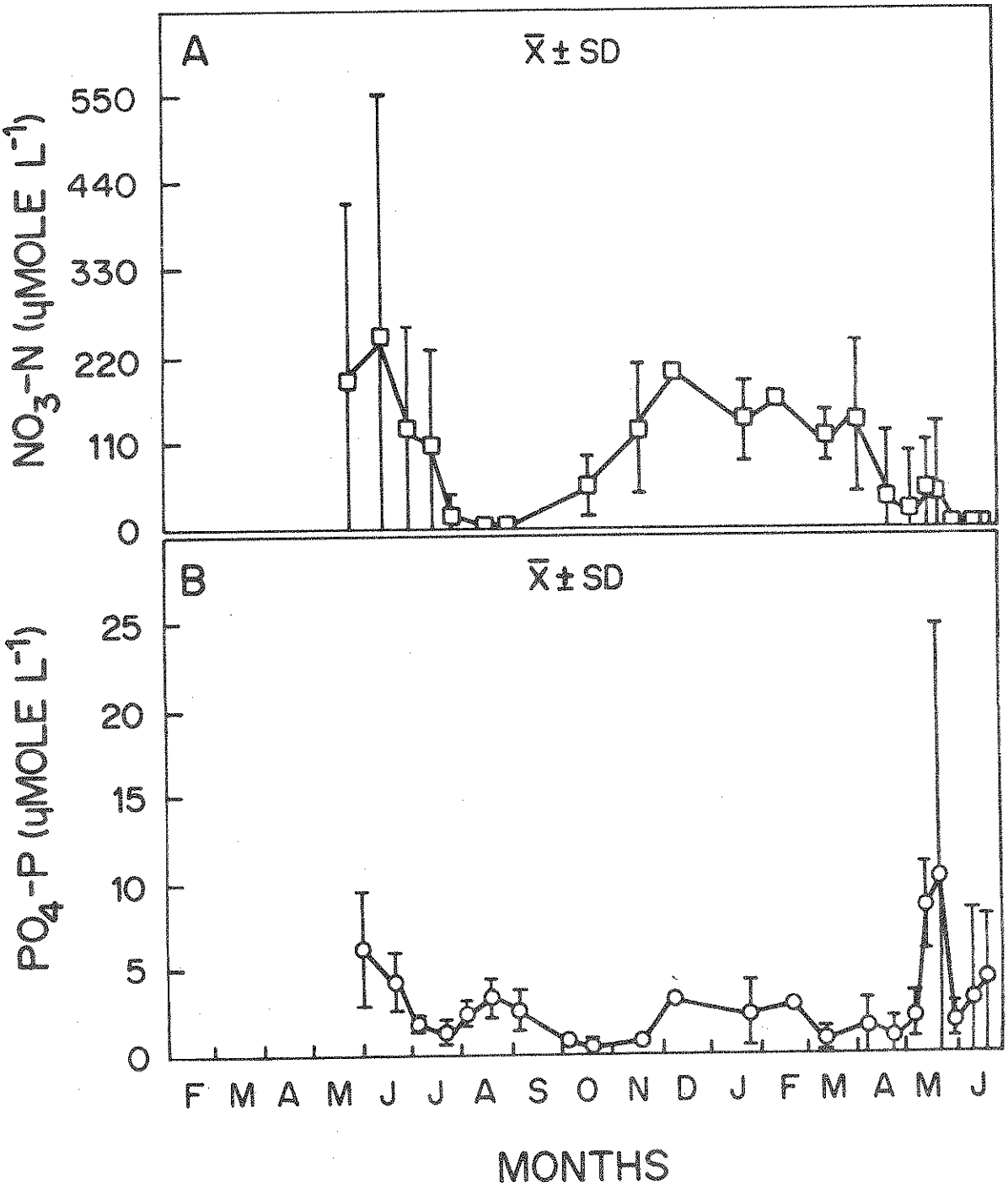


Fig. 33. Mean NO_3-N and PO_4-P concentrations in the cove region of Surrey Lake from May, 1978 - June, 1979.

Table 6. Percent germination of P. oedogonia akinetes under 10 and 13 h photoperiods at 20 C.

	Photoperiod (h)	
Sample	10	13
1	83	85
2	83	100
3	100	100
4	100	100
5	100	100
6	100	100
7	100	100
8	100	100
Mean	96	98
Total Akinetes Examined	117	119

Table 7. Germination of P. oedogonia akinetes at four temperatures, LD cycle = 10:14. Values given are X ± S.D.

Temperature	Medium	% Germination	Total Akinetes Examined
10	TBIM[a]	0 ± 0	422
	Cl(II)[b]	0 ± 0	169
	Surrey Lake Water[c]	0 ± 0[d]	75
12.5	TBIM	6 ± 10	51
15	TBIM	46 ± 21	168
20	TBIM	99 ± 1	353
	Cl(II)	47 ± 11	195
	Surrey Lake Water	100 ± 0	48

[a] Akinetes from laboratory cultures

[b] Akinetes from Surrey Lake, January, 1979

[c] Akinetes from Surrey Lake, February, 1979. NO_3-N = 167.06 μM; PO_4-P = 3.0 μM.

[d] After conclusion of the experiment, these akinetes were transferred to 20 C. 100% germination occurred after 7 days.

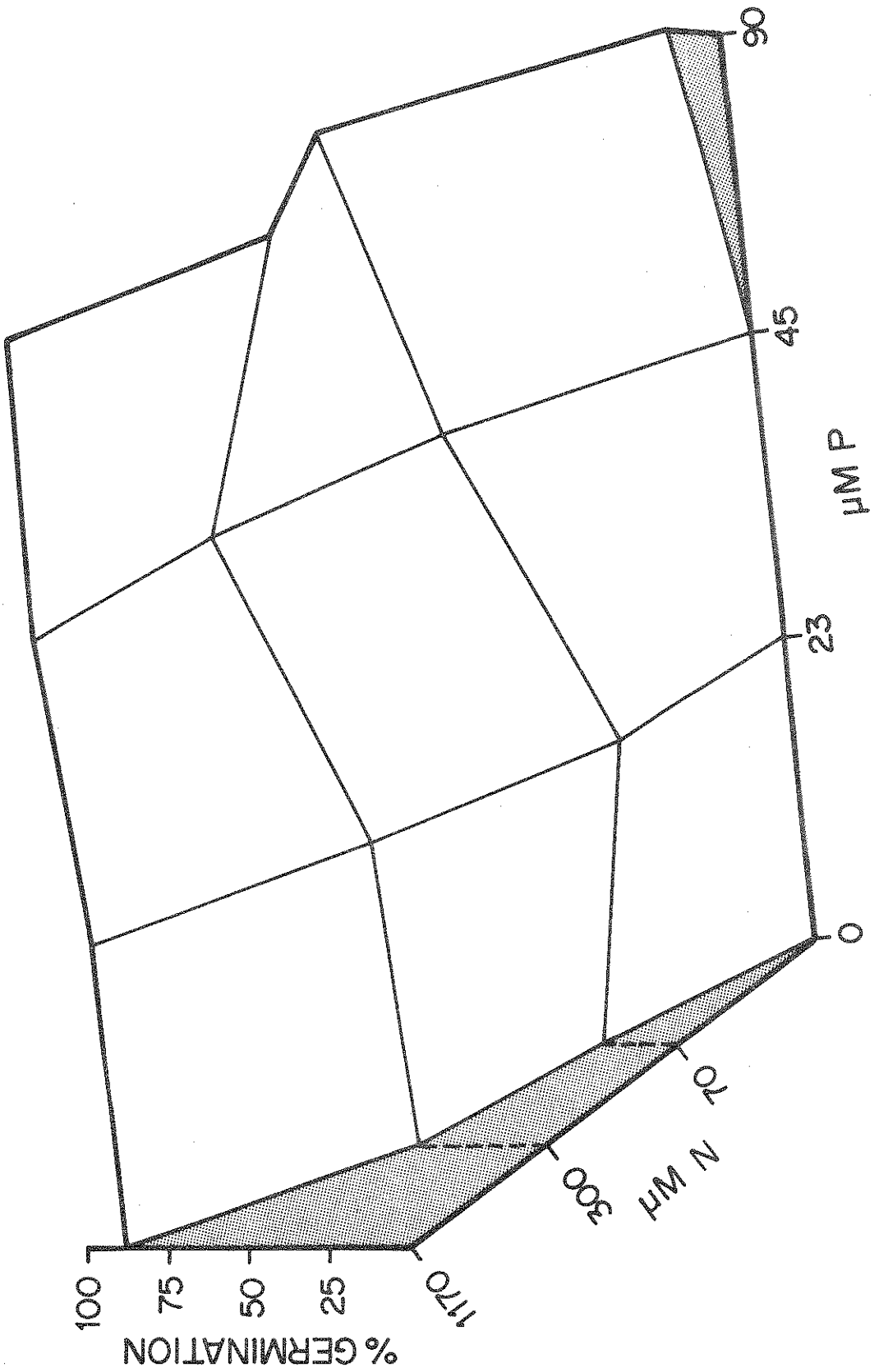


Fig. 34. Akinete germination as a function of external NO_3-N and PO_4-P concentrations in modified C1(II) medium.

Table 8. Results of two way analysis of variance and test for simple main effects for an experiment measuring akinete germination in response to NO_3-N and PO_4-P, including levels of significance (**, $P < 0.01$; NS = not significant at the 0.05 level).

Source of Variation	F	
PO_4-P	6.70	**
PO_4-P at 0 μM NO_3-N	1.50	NS
PO_4-P at 70 μM NO_3-N	7.38	**
PO_4-P at 300 μM NO_3-N	5.03	**
PO_4-P at 1170 μM NO_3-N	1.27	NS
NO_3-N	34.31	**
NO_3-N at 0 μM PO_4-P	9.53	**
NO_3-N at 23 μM PO_4-P	8.85	**
NO_3-N at 45 μM PO_4-P	15.65	**
NO_3-N at 90 μM PO_4-P	8.74	**
NO_3-N x PO_4-P	16.50	**

PO_4-P tested. Comparison of this value with mean NO_3-N and PO_4-P levels for water samples collected from the cove area of the lake (Fig. 33) indicates that the levels of these nutrients were generally high enough to support germination throughout the year especially during the winter months. If germination were being controlled only by these nutrients, then we would expect the akinete number to be lower in winter than in summer. Since akinete numbers were clearly greater in winter than summer, it is unlikely that changes in the concentrations of these nutrients were regulating the timing of germination in Surrey Lake.

An additional set of experiments was performed to test the importance of temperature as the factor controlling the timing of akinete germination. For these experiments, akinetes collected from Surrey Lake were incubated in concurrently collected Surrey Lake water at 10 and 20 C, with an LD cycle of 10:14. The akinetes and water were collected in February, 1979 when the N and P concentrations were relatively high (Table 7). Akinetes incubated at 20 C had achieved 100% germination after seven days, while those incubated at 10 C had not germinated (Table 7). To insure that the 10 C akinetes were viable, they were transferred (in the initial lake water) to 20 C. After 7 days, 100% germination occurred.

Further evidence that germination occurred in response to temperature comes from the timing of the germination episode for 1979. In 1979, the germination episode occurred in late April, approximately 3-4 weeks earlier than observed in 1978. Interestingly, the photoperiod at this time was shorter than the photoperiod associated with germination in 1978. NO_3-N and PO_4-P levels associated with germination were about the same for both years. In 1979 the water temperature had reached 22 C by April 22. In contrast, the water temperature did not reach this level in 1978 until between May 15-31, approximately 3-4 weeks later than in 1979.

The results of our experiments indicate that low temperatures inhibit germination of P. oedogonia akinetes even when other conditions such as nutrients and photoperiod are at levels suitable for germination, suggesting that akinetes in Surrey Lake germinate in response to rising water temperatures and not increases in photoperiod or external nutrient concentration.

Environmental Regulation of Akinete Formation. Akinete formation in Surrey Lake occurred between late August and late October in 1978 and between

mid-September and late October in 1979. As noted in the previous section, akinete numbers throughout the year are inversely related to water temperature and photoperiod. Nutrient concentrations, particularly nitrate-nitrogen are very low at or during the period of akinete formation. Although we have not conducted laboratory experiments to determine whether declining temperatures or photoperiod initiate or promote akinete formation, there does seem to be good evidence that the major causal factor is nutrient depletion. As far back as 1908, Ernst suggested that akinete formation was a result of nutrient depletion. Our own observation of Pithophora in culture is that akinetes form as the culture ages; in Cl(II) medium this process takes 6-8 weeks after the initial transfer of filaments to new culture medium. Filaments placed in BBM medium grow much more slowly than in Cl(II) medium but eventually do form akinetes 6-7 months after transfer. Akinetes form even under conditions of long photoperiod (14:10 L:D) and high temperatures (21-24 C) suggesting that akinete formation in the field does not occur because of decreasing photoperiod and temperatures (or, in other words, in preparation for winter).

It is likely that the critical nutrient regulating akinete formation is nitrogen rather than phosphorus. For example, phosphate-phosphorus concentrations in Surrey Lake actually increased during the fall 1978 period when akinetes were forming (compare Figs. 31 and 33) whereas nitrate-nitrogen concentrations decreased to nondetectable levels during this period. In addition, Riley and Anderson (1976) found that upon deleting major and minor nutrients from the culture medium, akinete formation occurred only in nitrogen-deficient medium.

Akinete Viability. The viability of akinetes collected from the free-floating mats at Surrey Lake was determined using the standard akinete germination test. Germination at all collection dates appeared to be excellent, in most cases averaging above 50% (Table 9). It is difficult to determine from this data whether akinetes undergo a period of dormancy or maturation following their formation. Akinete formation in 1979 occurred between September 19 and October 30. Percent germination does appear to gradually increase from early October to the end of November which may indicate that newly formed akinetes require a period of maturation before they will germinate. However, this period is short-lived since the germination of winter-collected akinetes ranges from 85 to 100%. Long-term dormancy does not seem to be the case with Pithophora akinetes.

Table 9. Germination of *P. oedogonia* akinetes collected from free-floating mats at Surrey Lake at various times during the year. Values given are X ± S.D.

Date Collected	% Germination	Total No. Akinetes Examined
21 Feb. 79	100 ± 0	48
20 June 79	95 ± 5	156
3 July 79	62 ± 31	64
5 Oct. 79	50 ± 23	89
19 Oct. 79	69 ± 35	224
1 Nov. 79	69 ± 22	120
8 Nov. 79	71 ± 27	225
27 Nov. 79	88 ± 21	264
7 Jan. 80	85 ± 12	223
24 Mar. 80	100 ± 0	161

Conclusions. Akinete numbers in the free-floating mats of Surrey Lake and the Mud Lake Channel show a definite temporal periodicity being highest in the winter and lowest during the summer period. At no time are akinetes completely absent from free-floating mats and they appear to be viable throughout the year. The majority of akinetes germinate in Surrey Lake in the spring in response to water temperature increasing to 20 C. Vegetative biomass is present throughout the year and, as expected, is highest in the summer months following the germination episode. Although biomass is lower in the winter and spring, significant amounts are present under the ice forming a thick layer on the surface of the hydrosoil. Akinete formation in the fall is probably stimulated by a decrease in nitrate-nitrogen in Surrey Lake.

Akinete Populations and Viability in the Hydrosoil

Lake and pond hydrosoil may serve as a storehouse for akinetes and provide a source of new filament growth each spring. The numbers, longevity, and viability of filamentous algal spores and zygotes in hydrosoil are virtually unknown presumably because of their small size and the difficulties involved in separating them from soil particles. Pithophora akinetes, on the other hand, are large enough to be readily distinguished from soil particles with the use of either a dissecting or compound microscope. They can be removed from a hydrosoil sample with a pair of fine forceps and easily checked for viability with the standard germination test.

Akinete Numbers and Viability. At the present time we have only partial data on the numbers of akinetes found in the hydrosoil. Four hydrosoil cores were taken from station 4 in Surrey Lake in December, 1979. The cores were frozen and cut into 1 cm segments along their length. The central portion of each segment (= 6-7 ml) was diluted in 50 ml water with 2 ml Tronic and sonicated for 20 min to disperse soil clumps. The solution was further diluted in 400 ml water and akinetes in 4, 1 ml aliquots were counted in a Sedgewick-Rafter counting chamber.

As expected, akinete numbers in the hydrosoil are very high (Table 10), the largest number recorded being 33×10^6 m^{-2} at the 4-5 cm depth in core number 3. An average of 11.9×10^6 akinetes m^{-2} was found in the uppermost layer of hydrosoil. This is the layer most likely to provide new filament growth to overlying water upon germination. Although variable from core to core, akinete numbers appear to decrease between 5 and 8 cm in depth.

Table 10. Akinete numbers at various hydrosoil depths in Surrey Lake. Cores collected from station 4, December, 1979. Values given are X = ± S.D.

Depth (cm)	Core#	Akinetes m^{-2} (X 10^6)			
		1	2	3	4
0-1		8.0 ± 2.6	15.2 ± 4.7	11.3 ± 6.1	12.9 ± 0.9
1-2		14.4 ± 1.8	19.6 ± 2.9	12.0 ± 3.4	21.3 ± 5.4
2-3		6.8 ± 1.9	17.7 ± 2.9	19.9 ± 4.2	16.1 ± 3.5
3-4		10.0 ± 4.2	26.1 ± 3.9	24.6 ± 6.3	23.0 ± 2.8
4-5		14.5 ± 1.4	24.1 ± 2.3	33.3 ± 1.2	16.9 ± 6.7
5-6		12.0 ± 4.1	8.9 ± 3.5	18.2 ± 5.9	3.0 ± 0.6
6-7		14.3 ± 6.8	2.4 ± 0.9	10.2 ± 3.5	0.5 ± 0.4
7-8		9.4 ± 5.3	3.9 ± 0.8	9.3 ± 3.0	2.3 ± 1.8
8-9		3.1 ± 0.5	20.0 ± 1.1	7.4 ± 2.3	1.3 ± 0.5
9-10		0.5 ± 0.4	21.3 ± 4.4	7.6 ± 4.9	2.9 ± 2.2
10-11		-	-	-	0.3 ± 0.3
11-12		-	-	-	0.6 ± 0.2
12-13		-	-	-	4.0 ± 1.5
13-14		-	-	-	-
14-15		-	-	-	1.0 ± 0.9

More cores are presently being collected to determine variability in the spatial and temporal distribution of hydrosoil akinetes in Surrey Lake.

An estimate of the potential for long-term viability in the hydrosoil was determined using Pithophora akinetes from culture. Akinetes were stored in test tubes in culture medium (BBM) in the dark and at 4 C. At intervals, 2-3 tubes were removed and placed under standard germination conditions. With the exception of the period between the second and third months of cold dark storage, loss in akinete viability was gradual (Fig. 35). After 3 years, viability was still at 19.6%.

To determine akinete viability in the hydrosoil of Surrey Lake, 2 soil cores were taken from station 2 in the shallow end of the lake in January and February, 1980. One cm length segments were taken from the central portion of the cores, spread out in petri plates and diluted with Cl(II) medium. After 2 days exposure to light and 21 C, akinetes became bright green in color and could be easily distinguished from the soil particles. Akinetes or akinete chains were removed from each plate with forceps, inoculated into Cl(II) medium and placed for another 5 days under standard germination conditions.

Akinetes in core #2 were found in high numbers from the surface to 9 cm in depth. Below that depth it was difficult to find akinetes for inoculation. Akinetes showed excellent germination to a depth of 10 cm (Table 11). Below 10 cm, germination occurred less frequently and of those that did germinate, germination tubes were abnormally stunted in comparison to the akinetes from the higher profiles.

Effect of Anaerobic Conditions on Akinete Germination. Hydrosoil under extreme reducing conditions can become anaerobic. The effect of anaerobiosis on akinete germination was evaluated by bubbling nitrogen gas through akinetes in culture medium (BBM) for 10 days. The experiment was conducted in flasks in the light at 24 C. At the end of this period, only 5.7% of the akinetes had germinated in contrast to aerated cultures in which 100% germinated. Air was then bubbled through the anaerobic flasks to restore aerobic conditions. After 5 days, 100% of the akinetes had germinated. This data clearly shows that germination is repressed when akinetes are placed under anaerobic conditions. Experiments are presently being conducted to determine the amount of dissolved oxygen required for akinete germination.

Conclusions. Extremely high numbers of akinetes are present at the surface and to depths of 5-8 cm in the hydrosoil. The majority of these

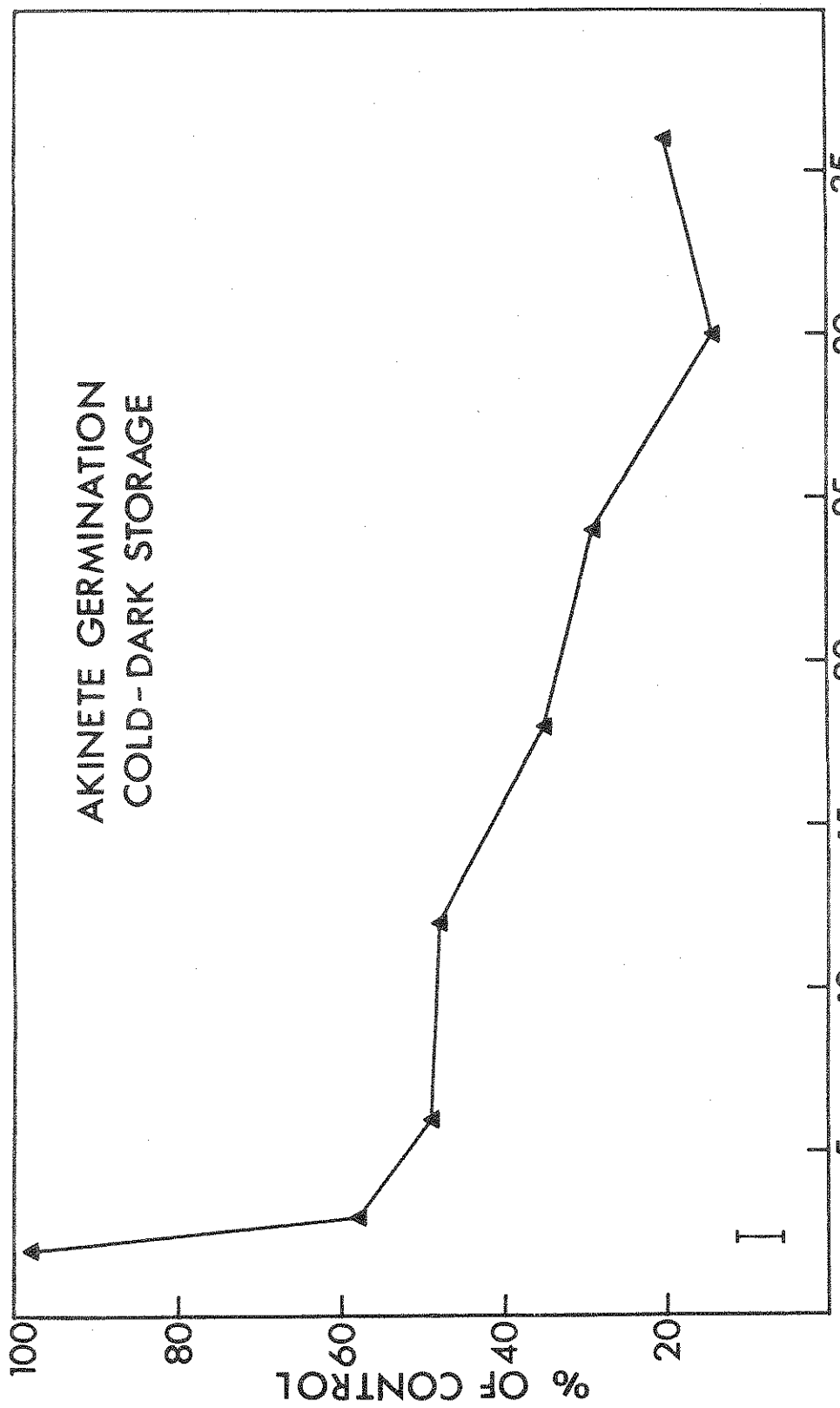


Fig. 35. Effect of cold-dark storage on akinete viability.

Table 11. Germination of P. oedogonia akinetes in hydrosoil cores taken from Surrey Lake. Cores collected from station 2, January and February, 1980.

	Core 1		Core 2	
Depth (cm)	No. of Akinetes[a]	% Germination	No. of Akinetes[a]	% Germination
0-1	20	95	20	100
1-2	20	95	24	100
2-3	20	100	32	100
3-4	11	100	26	100
4-5	20	80	25	100
5-6	20	85	40	100
6-7	20	85	30	93
7-8	20	100	26	85
8-9	-	-	31	100
9-10	-	-	20	100
10-11	-	-	10	40
11-12	-	-	21	48
12-13	-	-	4	0
13-14	-	-	10	50[b]

[a] No. of akinetes = number inoculated for germination test.

[b] Short germination stubs formed rather than normal elongate germination tubes.

akinetes have the potential for germination as long as aerobic conditions prevail (at the present time, we are assuming that temperature and nutrient requirements are the same for soil akinetes as for akinetes in free-floating mats). From a practical sense, it seems unlikely that akinetes at depths below 2-3 cm are ever presented with the opportunity to germinate unless the hydrosoil is disturbed. However, data on germination versus depth of collection are required to test this hypothesis. We also do not know the age of the akinetes at the greater soil depths. When the lake was constructed in 1945, the maximum water depth was 3.7 m. Today the maximum water depth is 2.7 m. These figures roughly indicate a sedimentation rate of 2.9 cm yr^{-1} in the deep end of the lake. The shallow end presumably fills in at a faster rate. A rate of 2.9 cm yr^{-1} would establish the age of the akinetes at the 10 cm depth (if they had been collected from the deep end) at 3.4 years. Thus, hydrosoil akinetes from stations 2 and 4 in the shallow end are probably even younger. The retention of viability for 2-3 years in the sediments is certainly not unreasonable particularly in light of the data obtained from our cold-dark storage experiment in the laboratory. Thus far, we have no evidence to suggest that akinetes can remain viable for long periods of time such as 10-20 years.

The key question that now remains to be answered is the role of hydrosoil akinetes in providing new growth in the spring (in relation to overwintering free-floating mats) or after chemical treatment or mechanical removal of the free-floating mats. Field experiments are currently being established to study this problem.

Akinete Tolerance to Adverse Environmental Conditions

If management methods other than the use of herbicides are to be attempted for the control of Pithophora (methods such as lake or pond drawdowns), then the environmental conditions under which akinetes will lose viability must be defined. In a drawdown situation, the most important stress parameters would be freezing and thawing activity in the winter, and desiccation, high temperatures and high light intensities in the summer.

In the laboratory experiments described below, akinetes were inoculated into culture media prior to exposure to stress conditions (except in desiccation experiments). After exposure, the culture tubes were placed under standard germination conditions and germination as % of control was

determined after 7 days. Akinetes collected from field trials were also placed in culture medium under standard germination conditions to determine % germination.

Akinete Survival under Summer Drawdown Conditions. Laboratory experiments were conducted to determine the tolerance of akinetes to high temperatures and to desiccation. Temperature experiments were conducted by placing akinete culture tubes in a circulating water bath. Akinetes were found to tolerate 1 hr exposure to 45 C (Fig. 36). The highest temperature tolerated after 12 and 24 hr exposure was 40 C. A very small percentage of akinetes survived 1 hr exposures to 50 and 55 C with no survival found at any time period after exposure to 60 C. Thus, temperatures above 40 C appear to be detrimental to akinete viability.

Akinetes from culture were also tested for their ability to withstand rapid desiccation. The medium surrounding clumps of akinetes was withdrawn with a pipette. The akinetes were observed with a dissecting microscope as they air-dried at room temperature. As the water evaporated, the contents of the akinetes were observed to collapse leaving a clear area or "bubble" just beneath the cell wall. As soon as collapse occurred, akinetes were transferred to liquid medium and tested for germination. When transferred at the time of initial collapse, only 7% of the akinetes germinated. By 5 and 10 min after collapse, none of the akinetes remained viable. Akinetes thus appear to be very susceptible to rapid drying conditions. This in itself might hold some hope for the success of drawdown as a control method.

Field trials simulating summer drawdown conditions were conducted in 1979. We had observed at Surrey Lake and at other Pithophora-infested areas that mats of the alga left stranded along the shoreline in late summer are often as much as 15-20 cm thick and, once the grayish surface layer (usually less than 0.5 cm thick) is pealed away, green. Thus, the majority of the mat may contain viable algal material.

Free-floating mats of Pithophora were collected from Surrey Lake on June 20, 1979. The mats were placed in 6 shallow pits each with a surface area of 1800 cm^2 and a depth of 10 cm. The thickness of the algal material was 8-10 cm. Within 3 days of placement, the surface layer of the mat had turned gray and appeared totally desiccated. At appropriate time intervals, algal samples were collected from below the gray layer from 4 of the 6 pits and transported to the laboratory in plastic bags. Each sample was divided

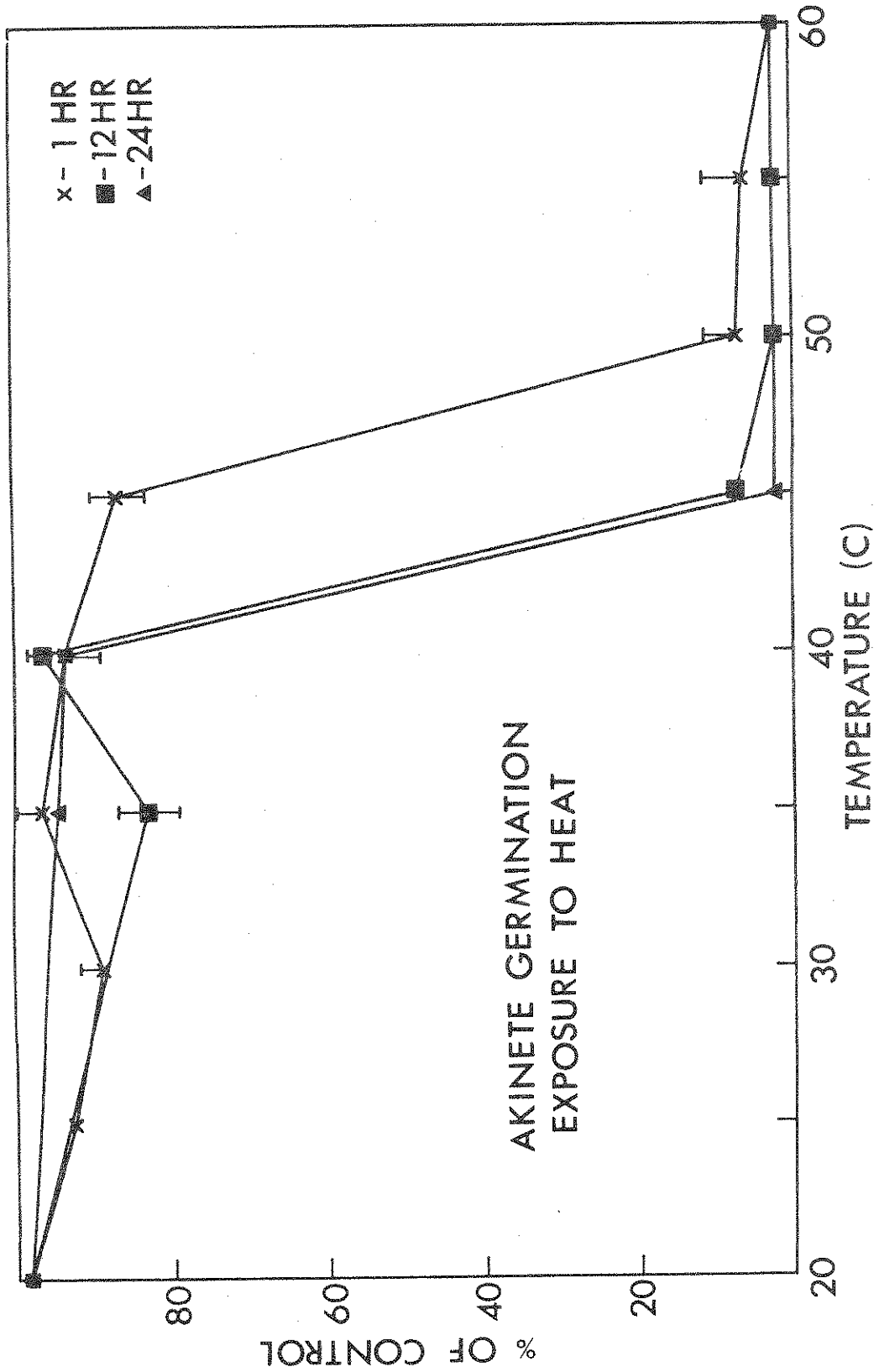


Fig. 36. Effect of high temperatures on akinete viability.

into 3 subsamples. One subsample was oven dried at 105 C for dry weight determinations. The second set of subsamples was combined, rinsed of adhering soil particles, blotted dry with a paper towel, and weighed. The sample was then disrupted in a Waring Blender in water at medium speed for 10 min. Akinete counts were taken on 4, 1 ml aliquots of the suspended material dispersed into Sedgewick-Rafter counting chambers. The number of akinetes g^{-1} fresh wt. (rewetted) was calculated. The third subsample from each pit was rewetted with Cl(II) medium and akinete chains were picked out and inoculated into Cl(II) medium for the standard germination test.

Samples were collected at weekly intervals from June 20 to July 27. Three other sampling dates were August 24, September 4, and November 7. By August, the mats were too heavily impregnated with soil washed into the pits during heavy rainstorms to obtain either accurate fresh and dry weight measurements or akinete counts. However, sufficient akinetes were present in the material to conduct viability tests.

Temperature measurements of the mat surface and mat interior approximately 4-5 cm within the mat) were taken as well as air temperatures (Fig. 37). Mat surface temperatures varied considerably but at most dates were above 40 C and well above air temperatures. At no time did internal mat temperatures reach 40 C. The maximum temperature recorded in the mats was 35 C on July 16 and August 7. This temperature is well within the range for the maintenance of viable akinetes.

Drying of the vegetation in the pits increased gradually over the first 4 weeks (Table 12). Heavy rains the evening before the 5 week sampling date lowered the % dry wt by almost half. The maximum dry weight over the period was 42.2% on July 20.

The most interesting finding other than the high viability of the akinetes throughout the summer and fall seasons (Table 12) was that akinete formation occurred rapidly following the placement of mats in the pits. At the time of placement, akinete counts (1500 g^{-1}) were reflective of the low numbers usually found in summer free-floating mats. Within a week of placement, cytoplasm was found to be concentrating at the tips of filament branches as the first stage in akinete formation. The rate of formation was most rapid the first 2 weeks after placement and then tapered off to reach a high of 113,000 akinetes g^{-1} by 5 weeks. This is a much higher akinete count than ever noted in the free-floating mats collected from the water.

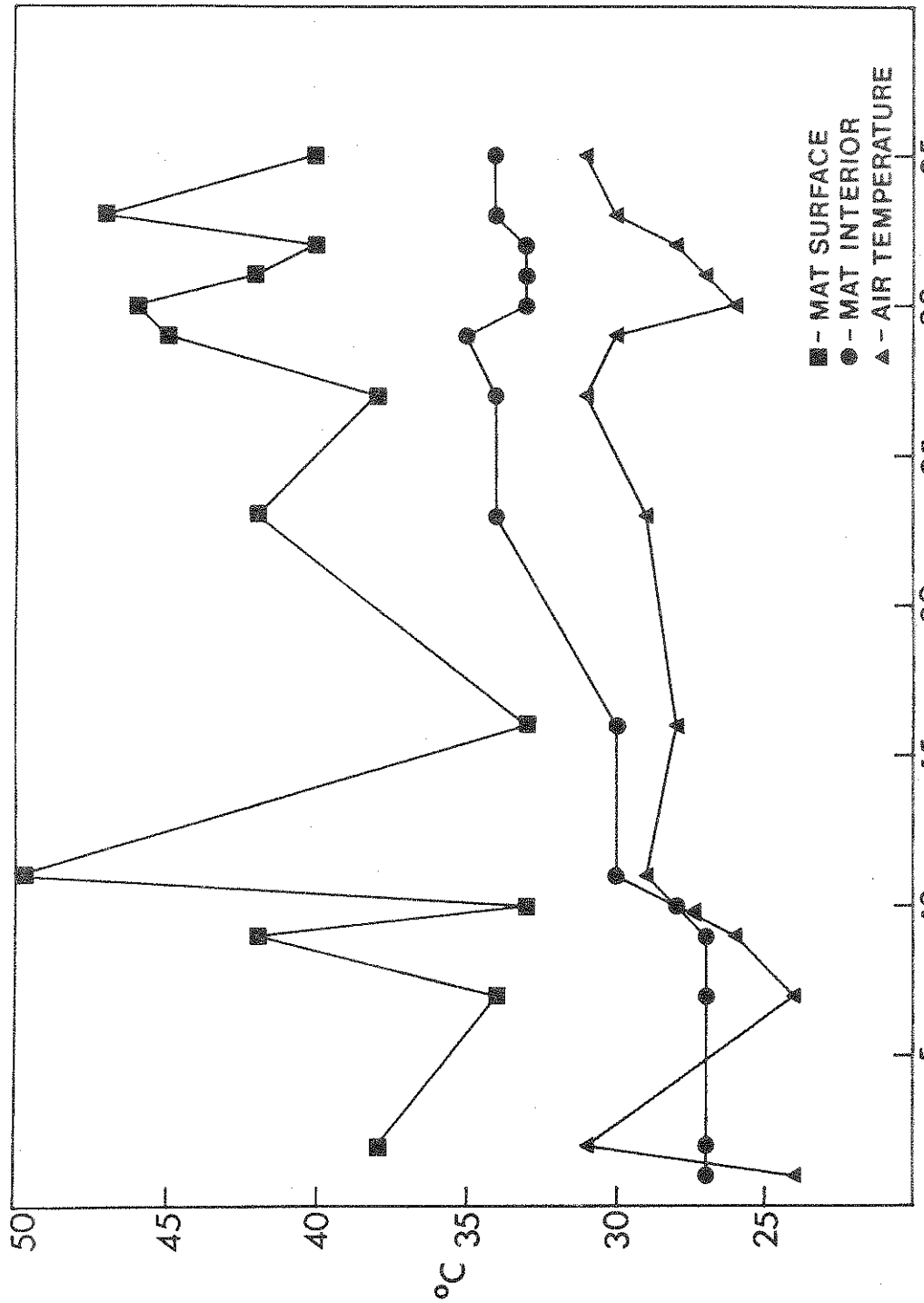


Fig. 37. Air, mat surface, and internal mat temperatures during simulated summer drawdown conditions.

Table 12. Akinete formation and viability in P. oedogonia mats subjected to simulated summer drawdown conditions. Mats placed in pits 20 Jun 79. Values given are X = ± S.D.

Days 20 Jun - 7 Nov	% Dry Wt.	Akinetes g^{-1} (X 10^3)	% Germination
0	13.5	1.5 ± 0.1	100 ± 0
7	17.3	11.5 ± 0.4	100 ± 0
14	27.7	63.6 ± 14.1	100 ± 0
21	38.9	88.2 ± 4.4	99.4 ± 1.2
28	42.2	92.0 ± 4.9	94.8 ± 3.1
35	23.0	113.3 ± 8.3	98.4 ± 1.5
65	-	-	91.7 ± 5.6
76	-	-	88.3 ± 5.0
136	-	-	80.3 ± 32.9
35 (Mat surface)	3.7	2.8 ± 0.1	0 ± 0

Akinete formation thus appears to be stimulated by the gradual desiccation of Pithophora mats.

At the 5 week date, samples of the dried surface layer (top 0.3 cm) were collected and subjected to the same tests as the underlying vegetation (Table 12). The % dry weight prior to the sampling date was probably much higher than that recorded, but due to heavy rains the night before, the % dry weight was only 83.7%. Akinete numbers were low and none of them were viable. Since the surface layers appear to dry out and bleach shortly after exposure to drying conditions, it appears that not enough time is available for akinete formation and that those akinetes that are present die either from desiccation or from high temperatures.

Since the underlying vegetation appears to be protected by the surface layer, it was of interest to determine whether disruption of the mats and exposure to atmospheric conditions would kill the akinetes that had formed. At the 4 week date, clumps of the underlying vegetation from the pits were removed and spread out on the soil surface. Samples were then checked for dry weight changes and viability.

The % dry weight increased over the first 5 h but decreased at 10 h due to the evening humidity (Table 13). Germination levels remained high until 48 h when they dropped to 4.4%. In order to determine if akinete mortality was due to desiccation or to high temperatures and light intensities, 6 week old material was collected from the pits. One sample was subjected to the high light and temperature conditions of the field whereas another sample was exposed to lower light and temperature conditions. The relative humidity in both cases was the same. The sample exposed to high light and temperature conditions lost most of its moisture by 1 h (Table 14). Even though relative humidity was high (91%), the sample was subjected to a brisk wind which probably hastened the drying process. Moisture loss in the sample placed under low light and temperature conditions was more gradual reaching 100% by 10 h. Although mortality was delayed under the low light and temperature conditions, the number of akinetes germinating by 48 h was very low. Variation in germination among akinetes subjected to the high light and temperature conditions is probably due to the protection afforded to akinetes by their clumping habit, even though we tried to spread the clumps out as evenly as possible before exposing them to the atmosphere. This data indicates that high light and temperatures may hasten akinete mortality, but

Table 13. Akinete viability following removal from mat under summer drawdown conditions. Max. irradiance = 800 μW cm^{-2}-nm; temp = 46 C; RH = 65%. Values given are X = $\pm$ S.D.

Hours	% Dry Wt.	% Germination
0	35.5	94.5 $\pm$ 8.2
1	64.9	80.8 $\pm$ 11.7
5	92.2	75.7 $\pm$ 16.5
10	74.0	73.5 $\pm$ 24.2
24	93.1	63.4 $\pm$ 18.7
48	96.5	4.4 $\pm$ 3.2

Table 14. Akinete viability under two light and temperature regimes following removal from mat.

	High[a]		Low[b]	
Hours	% Dry Wt.	% Germination	% Dry Wt.	% Germination
0	25.6	100.0	26.1	93.2
1	91.5	79.7	71.8	93.4
5	83.0	67.8	78.2	67.2
10	100.0	43.7	100.0	48.1
24	97.3	0.9	97.7	20.8
48	100.0	9.3	100.0	6.8

[a] Max. irradiance = 714 μW cm^{-2}-nm; temp = 46C; RH = 91%

[b] Max. irradiance = 0.49 μW cm^{-2}-nm; temp = 28C; RH = 91%

desiccation appears to be the most important factor.

Other tests with field collected material have been conducted to determine if a more gradual drying out period would increase akinete resistance to the effects of desiccation. Free-floating mats were collected on January 7, 1980, placed in enamel pans to a depth of 5 cm and allowed to gradually dry in the greenhouse. Again, a surface layer of dead filaments developed to prolong the drying of the internal portions of the mats. However, drying did occur throughout the mat over a 30 day period and by 30 days a marked decrease in viability was recorded (Table 15). By 50 days, no viable akinetes remained. It should be noted that the number of akinetes at the time of collection was in agreement with the high numbers recorded in midwinter samples and that again, as in the field, an increase in akinete numbers was observed.

Akinete Survival under Winter Drawdown Conditions. Laboratory experiments were conducted to indicate the degree of akinete susceptibility to freezing and freeze-thaw cycles. During these tests, all akinetes were frozen in their culture medium (BBM) and allowed to thaw in that medium.

Prolonged exposure to -10 C produced a gradual loss in viability over a period of 14 days (Fig. 38). By 30 days of continuous freezing, no akinetes remained viable. Freeze-thaw regimes of 12-12 h produced even more severe effects on the akinetes (Table 16). For example, after 4 cycles of freezing and thawing (total of 48 h at -10 C), only 17% of the akinetes as compared to control cultures were viable whereas 55% of those under continuous freezing conditions for 48 h were still viable. These experiments seem to indicate that akinetes might be more susceptible to a winter rather than to a summer drawdown.

Field studies included viability checks of frozen akinetes from Surrey Lake and simulations of winter drawdown conditions. Akinetes from Surrey Lake were collected from either lake ice that was allowed to thaw or from mats that were stranded above the shoreline over the winter period. Although there is variation in the data, germination of akinetes collected from Surrey Lake was excellent at most of the sampling dates (Table 17). The mats sampled in April, 1979 and March, 1980 had been exposed through the entire winter as well as the early spring freezing and thawing period.

Shallow pits were constructed on November 8, 1979 and filled with Pithophora mats from Surrey Lake to a depth of 4-5 cm. The % dry weight

Table 15. Germination of P. oedogonia akinetes upon exposure to gradual drying conditions. Plants collected from Surrey Lake January, 1980. Values given are $X \pm$ S.D.

Days	% Dry Wt.	Akinetes g^{-1} ($X10^3$)	% Germination
0	19.2	18.8 ± 2.0	84.5 ± 11.7
7	29.8	50.5 ± 0.1	99.6 ± 1.2
14	49.5	52.9 ± 0.5	98.5 ± 4.2
30	95.2	-	34.6 ± 22.9
50	96.9	-	0 ± 0

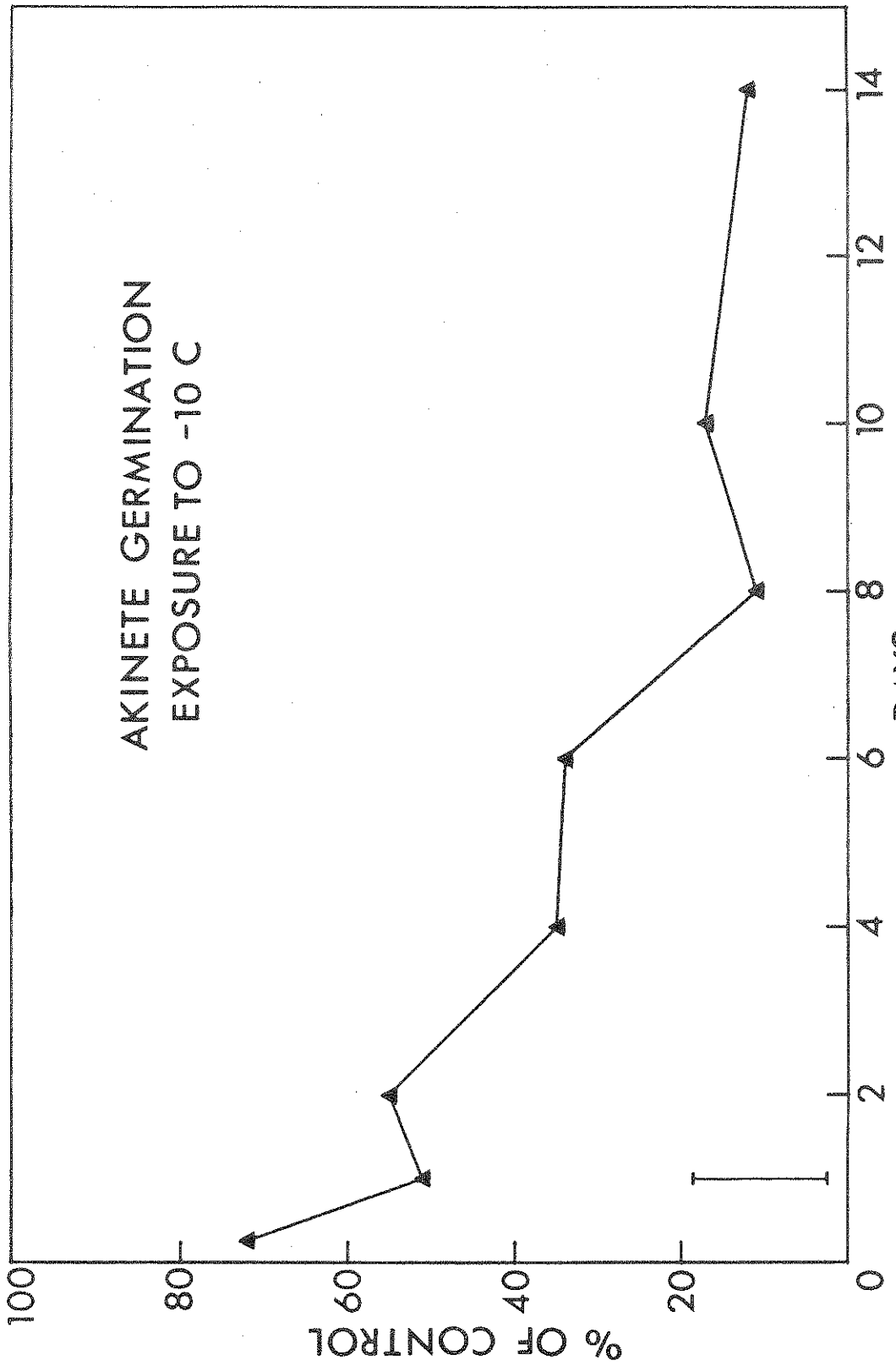


Fig. 38. Effect of exposure to -10 C on akinete viability.

Table 16. Effect of freeze-thaw (12-12 h) cycles on akinete germination.

No. Cycles	-10C (h)	Germination % of Control	
		Freeze-Thaw	Freeze only
1	12	50	51
2	24	34	51
3	36	24	-
4	48	17	55
5	60	9	37

Table 17. Germination of P. oedogonia akinetes exposed to freezing conditions at Surrey Lake. Values given are X ± S.D.

Date	Site	% Germination
29 Jan 79	Lake Ice	82.3 ± 4.9
21 Feb 79	Lake Ice	66.3 ± 4.8
6 Feb 80	Lake Ice	100 ± 0
21 Feb 79	Shoreline	61.6 ± 9.7
9 Apr 79	Shoreline	99.1 ± 0.6
19 Dec 79	Shoreline	88.1 ± 17.3
7 Jan 80	Shoreline	100 ± 0
25 Mar 80	Shoreline	100 ± 0

Table 18. Akinete formation and viability in P. oedogonia mats subjected to simulated winter drawdown conditions. Mats placed in pits 8 Nov 79. Values given are X ± S.D.

Days 8 Nov - 16 Mar	% Dry Wt.	Akinetes g^{-1} (X 10^3)	% Germination
0	—	19.7 ± 1.7	71.0 ± 27.0
10	17.5	43.7 ± 1.2	82.3 ± 26.4
20	18.5	45.5 ± 6.3	80.6 ± 29.1
30	21.3	40.1 ± 10.5	99.0 ± 1.8
40	19.7	24.2 ± 0.4	100 ± 0
130	—	48.5 ± 16.5	99.6 ± 3.7

(Table 18) did not change significantly over the winter period due to heavy rainfall in November and December. Although air and mat surface temperatures from November to mid-January fell to as low as -5 C, internal mat temperatures remained at 1 C or above. However, on January 23 (76 days after placement) when an air temperature of -12 C was recorded, internal mat temperatures had dropped to -1 C and the entire bulk of the mat remained frozen from that date until March 14 (128 days after placement). During most of this period, the pits were also covered with 5-8 cm of snow.

Akinetes appeared to form in the mats within 10 days after placement (Table 18). However, the approximately 2 fold increase in akinete number was much lower compared to the 10 fold increase in akinete numbers monitored during the simulated summer drawdown conditions (Table 12). It is quite possible that colder temperatures depress metabolic activities enough to prevent active akinete formation. The most important finding of this study, however, was that akinetes remained viable throughout the winter; in fact, percent viability may actually have increased as the winter progressed.

Conclusions. Our experiments indicate that akinetes are extremely susceptible to desiccation even when allowed to dry gradually over an extended period of time. Spores of other algae have been noted to survive prolonged periods of desiccation; for example the spores of blue-green algae such as Nostoc muscorum and Nodularia harveyana resumed growth after being stored under extreme desiccating conditions for 70 years (Bristol, 1919). Akinetes of a green filamentous alga, Oedocladium, survived 3.5 years of desiccation (Davis, 1970). However, all of these organisms are soil algae which would be expected to adapt to periods of prolonged desiccation. Akinetes of Zygnema and zygotes of Spirogyra, both green filamentous algae of ponds and lakes have been reported to survive desiccating conditions; in the case of Zygnema akinetes, for 12 months (McLean and Pessoney, 1971). This does not appear to be the case with Pithophora.

When Pithophora is stranded along the shoreline during the summer or, presumably, during a summer drawdown, the surface layer of cells is sacrificed to protect the underlying mats from desiccation and high temperatures. As a reaction to gradual desiccation (or possibly from nutrient depletion?), akinetes are formed in large numbers. Although these stranded mats would eventually dry out completely under very arid conditions, the frequency of rains in the midwest and southeast make it highly unlikely that Pithophora

mats would ever desiccate sufficiently to cause significant akinete control. Rainfall amounts during our field trials from the end of June to the first part of November were only slightly above normal rainfall levels for the west central portion of Indiana: 34.5 cm compared to a normal of 32.6 cm. In fact, the month of September was the driest on record with no rainfall at all recorded and yet only 20% of the akinetes in our field trial had lost viability by November.

Although laboratory experiments indicate a high degree of susceptibility to freezing conditions, akinetes in the field are tolerant to prolonged periods of freezing temperatures. Of relevance here is that blue-green algal (Fogg et al., 1973) and bacterial and fungal (Sussman and Halvorson, 1966) spore survival has been found to be greatly enhanced when the cultures are slow cooled rather than rapidly cooled. The slower rate of cooling permits the gradual condensation of the cytoplasm and the removal of much of the intercellular water thus preventing the formation of ice crystals in the cytoplasm. Field akinetes exposed to conditions of gradual cooling thus would be expected to show a better survival rate than laboratory akinetes which were subjected to a rapid decrease in temperature, i.e., from 21 C to -10 C within one hour. In this state, too, the akinetes would be much more able to tolerate alternating periods of freezing and thawing, conditions which are likely to occur in Indiana in early spring.

IV. NITROGEN AND PHOSPHORUS REQUIREMENTS FOR VEGETATIVE GROWTH

Although future control schemes for Pithophora must take akinete periodicity and tolerance to stress conditions into account, nutrient limitation must also be considered as an integral part of a management control program. Since the factors regulating vegetative filamentous growth in Pithophora were unknown, we conducted a combined laboratory-field study to determine those nutrient conditions which promote luxurient growth.

Materials and Methods

Surrey Lake is a small, shallow (Table 19) man-made lake in Bartholomew Co., central Indiana (T9N, R6E). The lake was constructed in 1945 and has supported extensive growths of Pithophora oedogonia since at least the late

Table 19. Morphometric and geographic characteristics of Surrey Lake, Indiana.

Parameter	
Maximum length	500 m
Maximum width	124 m
Area	3.9 hectares
Volume	5.5×10^4 m^3
Maximum depth	2.7 m
Relative depth	1.4 m
Shoreline	1428.9 m
Shoreline development	2.05
Longitude	85° 51' 17"
Latitude	39° 12' 18"

See Wetzel, 1975 for details of calculations.

1960's. Water flow is from south to north (Fig. 39). The inflowing stream drains a watershed of approximately 1128 ha, 62% of which is agricultural land (corn and soybeans) and pastures; 21%, woodland; and 16%, residential. Most of the residential area is within the boundaries of Jewel Village which is located 1.2 km upstream from Surrey Lake. The homes in Jewel Village are each on a septic tank-finger system and do not presently meet existing state codes for sewage disposal. The septic systems are located between the homes and the stream and probably contribute significantly to the high nutrient load in Surrey Lake. The major soil types in the Jewel Village drainage area are the Fincastle silt loam and Brookston silty clay loam which are indicative of poorly drained soils such that heavy rains may transport sewage material to the stream.

Sampling stations 1 through 12 were established along the length of Surrey Lake (Fig. 39). Stations 1-7 are located in the shallow or cove end of the lake and average 0.42 m in depth. Stations 8-12 are located in the "deep" end of the lake and average 2.0 m in depth. There is an outflow through a spillway at the dam or north end of the lake.

Water samples and algal biomass samples were collected from stations 2-12, Surrey Lake from May, 1978 to December, 1979 (station 1 was omitted as it was frequently inaccessible). Water samples were returned to the laboratory and analyzed for total nitrogen, nitrates, ammonia, and soluble organic nitrogen according to the techniques described by Nelson and Sommers (1972). Total phosphorus and soluble reactive phosphorus were determined using the ascorbic acid technique (Am. Publ. Health Assoc., 1971). Specific conductance and temperature were recorded in situ with a YSI Model 33 S-C-T meter; specific conductance was temperature corrected using the table provided in Wetzel and Likens (1979). Dissolved oxygen was measured with a YSI Model 54 Oxygen Meter. Sample pH was measured within 2 h of collection and total alkalinity determined by potentiometric titration with 0.02 N sulfuric acid to an endpoint of pH 4.5. Hardness and calcium were determined by EDTA titration as described by Lind (1974). Dissolved silica (SiO_2) was determined on filtered samples using the technique of Rainwater and Thatcher (1960).

Algal biomass samples were collected using a 30.48 cm diameter aluminum tube. The tube was inserted into the sediment and both floating and submerged algal filaments were collected. In the deeper part of the lake only the floating mats were sampled. Samples were returned to the laboratory, rinsed

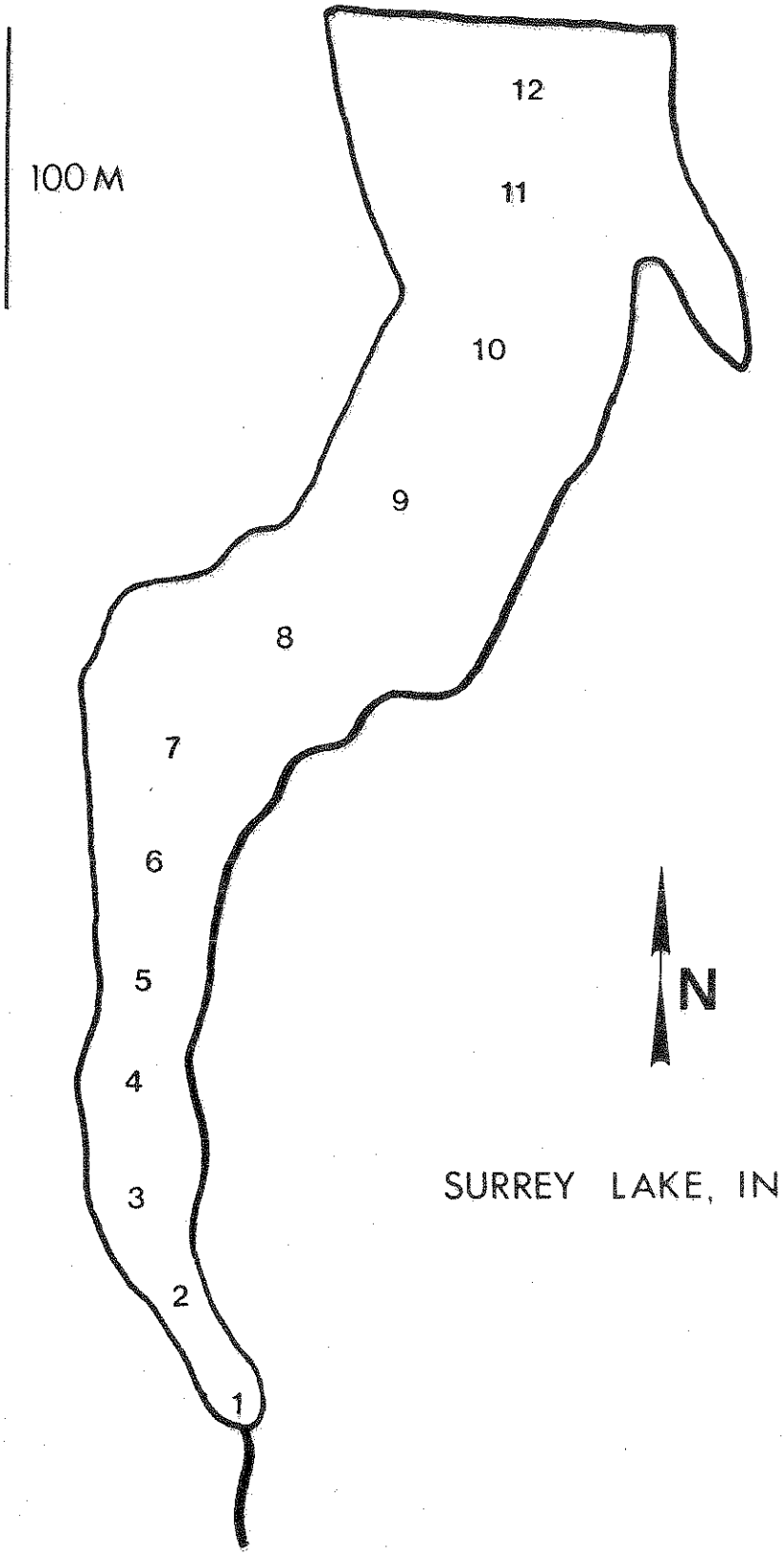


Fig. 39. Map of Surrey Lake, Indiana and approximate locations of sampling stations 1-12.

to remove sediments and Lemna, placed in aluminum trays and dried for 48 h at 110 C.

Culture studies were conducted in growth chambers set at a 16:8 L:D cycle, 20 $\pm$ 1 C, and 12 μeinsteins m^{-2} s^{-1}. Pithophora oedogonia was grown in C1(II) medium modified by deletion of the B7 and C13 trace element components (Gerloff and Fitzgerald, 1976). Fifty ml of medium was dispensed into 250 ml flasks, autoclaved, and inoculated with a small clump of P. oedogonia. The inoculation procedure is similar to that described by Gerloff and Fitzgerald (1976). In this procedure algal material from 5-6 week old cultures were cut into small clumps which were placed in ceramic spot plates. Care was taken to obtain equal sized inocula. The flasks were inoculated at random. Several clumps were blotted dry and the chlorophyll extracted in 90% acetone. Phaeophytin corrected chlorophyll concentrations were determined (Wetzel and Westlake, 1974). This gave an estimate of the biomass present at the beginning of the experiment. After 21 days, biomass was again determined by this technique. Growth rates (doublings day^{-1}) were determined (for each flask) using a linear regression of biomass (transformed to base 2) versus time. Using these data, the Michaelis-Menton parameters, K_s and V_{max}, were determined by a curvilinear regression technique described by Hanson, et al. (1967).

In July, 1979, two enclosures (made of 6.35 mm mesh screening) were placed at sites 3 and 10 in Surrey Lake. Pithophora collected from the lake was placed in the enclosures. Seven, fourteen, and seventeen days later samples of the algal material were collected, returned to the laboratory, and analyzed for alkaline phosphatase activity (Fitzgerald & Nelson, 1966) and dark uptake of ammonia (Fitzgerald, 1968). These procedures have been used as measures of algal nutritional status with regard to phosphorus and nitrogen, respectively. The procedures were as described except that C1(II) medium was used in place of Gorham's medium.

Results and Discussion

During May-November 1979, mean Pithophora biomass was 93 g $^{-2}$ dry weight. In addition, average Pithophora biomass was greater at stations 2-7 than at stations 8-12. Examination of (Fig. 40) reveals that prior to July 11 Pithophora was found only in the cove region (stations 1-7). After this time it occurred in the deeper part of the lake, but this can likely be explained

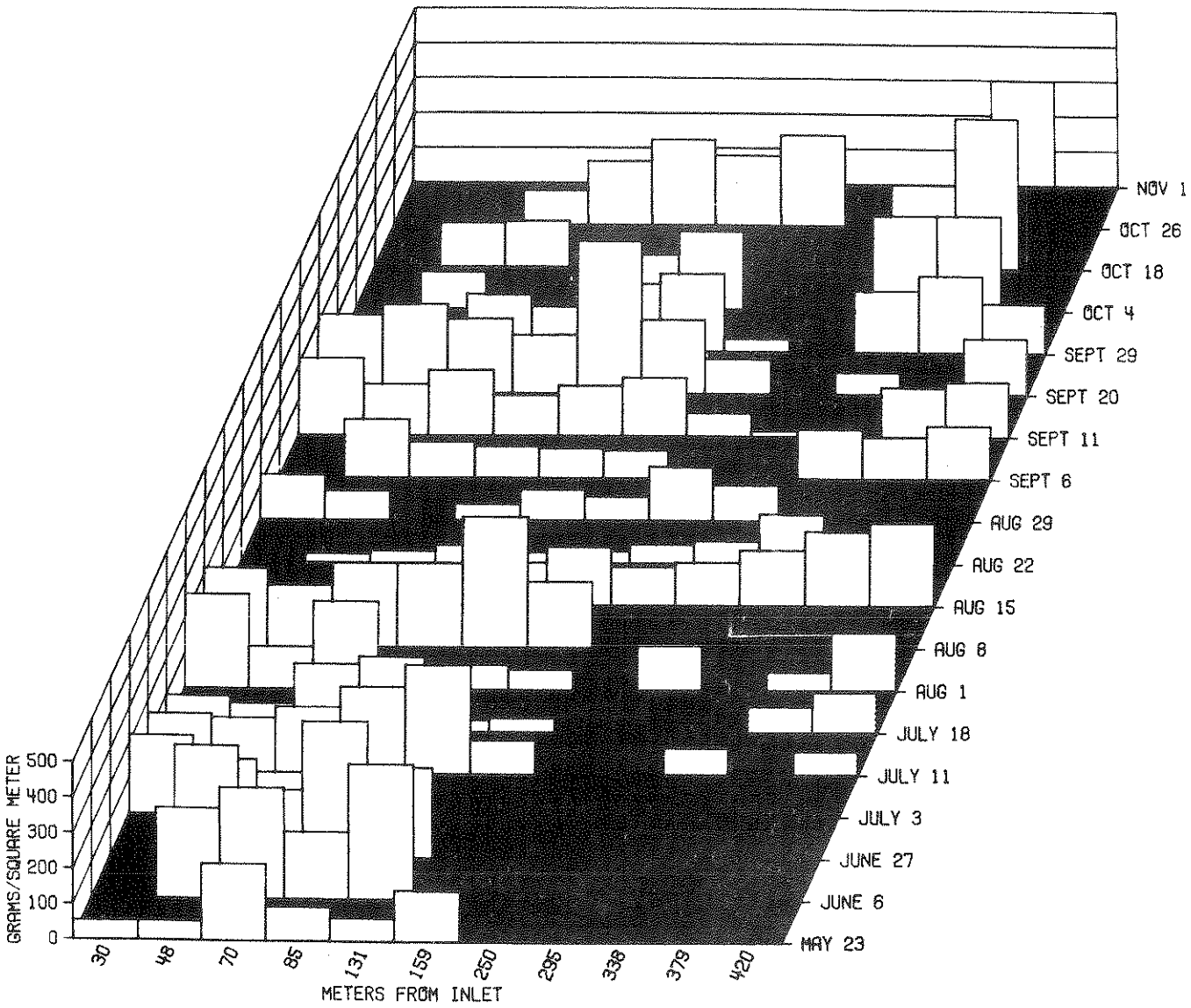


Fig. 40. Distribution of Pithophora biomass (g m^{-2} dry wt) in Surrey Lake from May - November, 1979. 30 m = station 2; 48 m = station 3, etc.

by wind induced movement from the cove. The prevailing winds are from south to north in the summer. We believe this accounts for the relatively high biomass at stations 11 and 12 later in the season.

Mean values for a number of Surrey Lake water quality parameters indicate the lake to be a hardwater eutrophic lake (Table 20). Examination of the concentrations of some important algal nutrients show interesting differences between the cove (stations 1-7) and open water part of the lake (stations 8-12) (Table 21). Mean concentrations of soluble reactive phosphorous (SRP) did not differ significantly between the cove and lake sampling stations. Similarly, neither did the mean ammonia concentration. This was not the situation, however, with respect to mean nitrate concentration. Nitrate concentration in the cove was nearly sixfold that of the open water part of the lake. When the nitrate data are presented graphically (Fig. 41), there is a clear inverse relationship between nitrate concentration and distance of the sampling point from the stream inflow. Because nitrogen and phosphorus are most frequently the nutrients limiting algal growth (Wetzel, 1975) and because of the apparent correlation between the distribution of Pithophora biomass and the concentration of nitrate but not SRP, we hypothesized that nitrates were important in regulating the distribution of Pithophora in Surrey Lake. To test this hypothesis we conducted laboratory growth experiments with Pithophora.

Monod (1950) proposed that growth of microorganisms could be related to external nutrient supply in terms of the following equation:

$$u = u_{max} \cdot \frac{uC}{K_s+C}$$

where

u = is the growth rate, u_{max} is the maximum growth rate, C is the concentration of the nutrient in shortest supply, and K_s is the half saturation constant relating growth to nutrient C, i.e., the concentration of C which supports half maximal growth. Epply et al. (1969) suggested that half saturation constants may be useful in explaining the distribution of planktonic algal species. Titman (1976) and Tilman (1977) demonstrated that this approach could explain 74% of the variance in the relative abundance of two species of Great Lakes diatoms. Therefore, we performed growth experiments with Pithophora oedogonia in culture with the purpose of calculating half saturation constants relating growth to external concentrations of nitrate and

Table 20. Mean values for Surrey Lake water quality parameters, May 15, 1978 - Sept. 7, 1979

Parameter	Mean $\pm$ S.E. ($mg\ l^{-1}$)	No. of Samples	Coefficient of variation
Total Phosphorus	0.232 ± 0.010	190	63%
Soluble Reactive Phosphorus	0.086 ± 0.010	215	106%
Ammonia-nitrogen	0.135 ± 0.008	215	83%
Nitrate-nitrogen	0.742 ± 0.086	215	171%
Keldahl Nitrogen	2.20 ± 0.11	186	68%
Calcium	41.9 ± 1.57	39	23%
Hardness*	150.3 ± 4.73	45	21%
Dissolved Silica	4.7 ± 0.60	44	85%
Alkalinity*	133.2 ± 2.67	158	25%
pH	8.3 ± 0.06	162	10%
Specific Conductance (μmhos)	412.4 ± 6.82	197	23%
Dissolved Oxygen	9.0 ± 0.38	187	57%
Temperature (C)	21.0 ± 0.50	213	32%

*$mg\ l^{-1}$ as $CaCO_3$

ble 21. Mean values for Surrey Lake water quality parameters at shallow (1-7) and deep ter stations (8-12), May 15 - Oct. 7, 1978 and June 26 - Sept. 7, 1979.

tes	Parameter	Number of Samples	Mean ± S.E. ($mg\ l^{-1}$)	T-Value	Degrees of freedom	2-Tailed Probability
12 7	Soluble Reactive Phosphorus	54 61	0.083 ± 0.008 0.112 ± 0.016	-1.53^{S}	88.9	0.130
12 7	Ammonia-nitrogen	54 61	0.112 ± 0.017 0.136 ± 0.013	-1.116^{P}	113	0.269
12 7	Nitrate-nitrogen	54 61	0.156 ± 0.049 0.880 ± 0.241	-2.94^{S}	68.8	0.005
12 7	Calcium	15 13	40.6 ± 1.6 42.9 ± 2.7	$-.071^{P}$	26	0.486
12 7	Silica	18 14	3.2 ± 0.8 5.7 ± 1.5	-1.60^{P}	30	0.121
12 7	Hardness	16 16	124.8 ± 3.7 163.3 ± 7.7	-3.33^{S}	21.69	0.003
12 7	Total Alkalinity	37 48	109.5 ± 4.2 139.3 ± 4.5	-4.70^{P}	83	0.000
12 7	Dissolved Oxygen	46 52	8.3 ± 0.4 5.9 ± 0.7	3.00^{S}	73.35	0.004
12 7	Temp (C)	54 57	24.2 ± 0.6 23.8 ± 0.6	0.41^{P}	109	0.683
12 7	pH	40 49	8.5 ± 0.1 7.9 ± 0.1	3.63^{S}	75.7	0.001
12 7	Conductivity (μmhos)	48 49	335 ± 6.1 464 ± 15.3	-7.88^{S}	62.7	0.000

1 The null hypothesis that $u_1 = u_2$ was tested using $t_{d^-} = \frac{(X_1 - X_2)}{S_{d^-}}$ with $(n_1 + n_2 - 2)$ df.

1 Sample variances heteroscedastic as determined by F_{max}. t approximated by

$$t = \frac{(\overline{X}_1 - \overline{X}_2)}{S_1^2/n_1 + S_1^2/n_2}$$

with $$df = \frac{(S_1^2/n_1) - (S_2^2/n_2)^2}{(S_1^2/n_1)^2/(n_1-1) + (S_2^2/n_2)^2/(n_2-1)}$$

See Nie et al., 1975, p 269-270 for details.

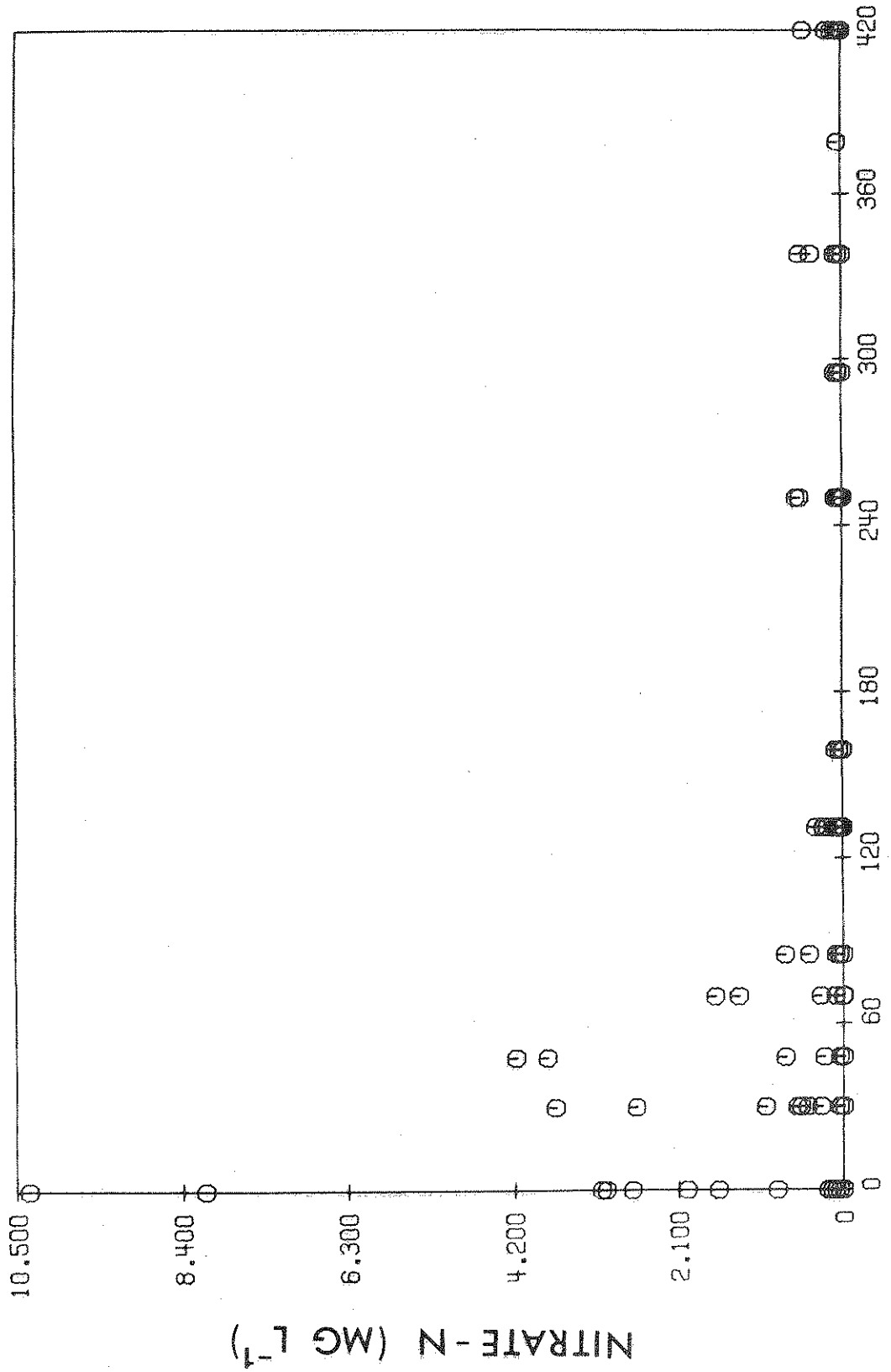


Fig. 41. Nitrate-N concentrations in Surrey Lake for May 15 - October 7, 1978 and June 26 - September 7, 1979.

phosphate. If our hypothesis about the importance of nitrate to Pithophora distribution in Surrey Lake were correct, we should expect the half saturation constants to be useful in predicting the distribution patterns observed in the lake.

Pithophora growth versus external nitrate and phosphate concentrations was determined separately, and could be fitted by a rectangular hyperbola (Figs. 42, 43). The K_s value for nitrate limited growth was 88 μM, whereas that for phosphorus limited growth was 3.2 μM (Table 22).

The relative importance of these nutrients can be assessed in the following way. Titman (1976) has suggested that growth of a species will be equally limited by two nutrients when the following equation holds

$$S_1/S_2 = K_1/K_2$$

where S_1 and S_2 are the concentrations of nutrients 1 and 2 and K_1 and K_2 are the half saturation constants relating growth to these nutrients. In the present case, Pithophora would be limited by both nitrate and phosphate when

$$NO_3\text{-}N/PO_4\text{-}P = 88/3.2 = 27.6$$

Accordingly when the NO_3:PO_4 ratio > 27.6, growth should be limited by phosphorus; at NO_3:PO_4 ratios < 27.6 growth should be limited by nitrate concentration.

Using the facilities of the SPSS computer programs (Nie et al., 1975), NO_3-N:PO_4-P ratios were calculated for each sampling station from May 15 - Oct. 7, 1978 and June 26 - Sept. 17, 1979. The results of this analysis clearly show that as the distance of the sampling point from the stream inflow increases, the probability of encountering a nitrogen limited environment also increases (Fig. 44), a finding which supports our earlier idea about the importance of nitrate in this lake.

These results are, however, based in part on the results of laboratory growth experiments. To further test the hypothesis concerning the importance of nitrogen we devised a set of field experiments. These experiments involved establishing enclosures in Surrey Lake that would allow the free movement of water but restrict the movement of Pithophora placed inside. One enclosure was placed in the cove (near station 3) and another in the open water part of the lake (near station 10). We reasoned that Pithophora exposed to ambient nutrient concentrations at these two locations should show differences in

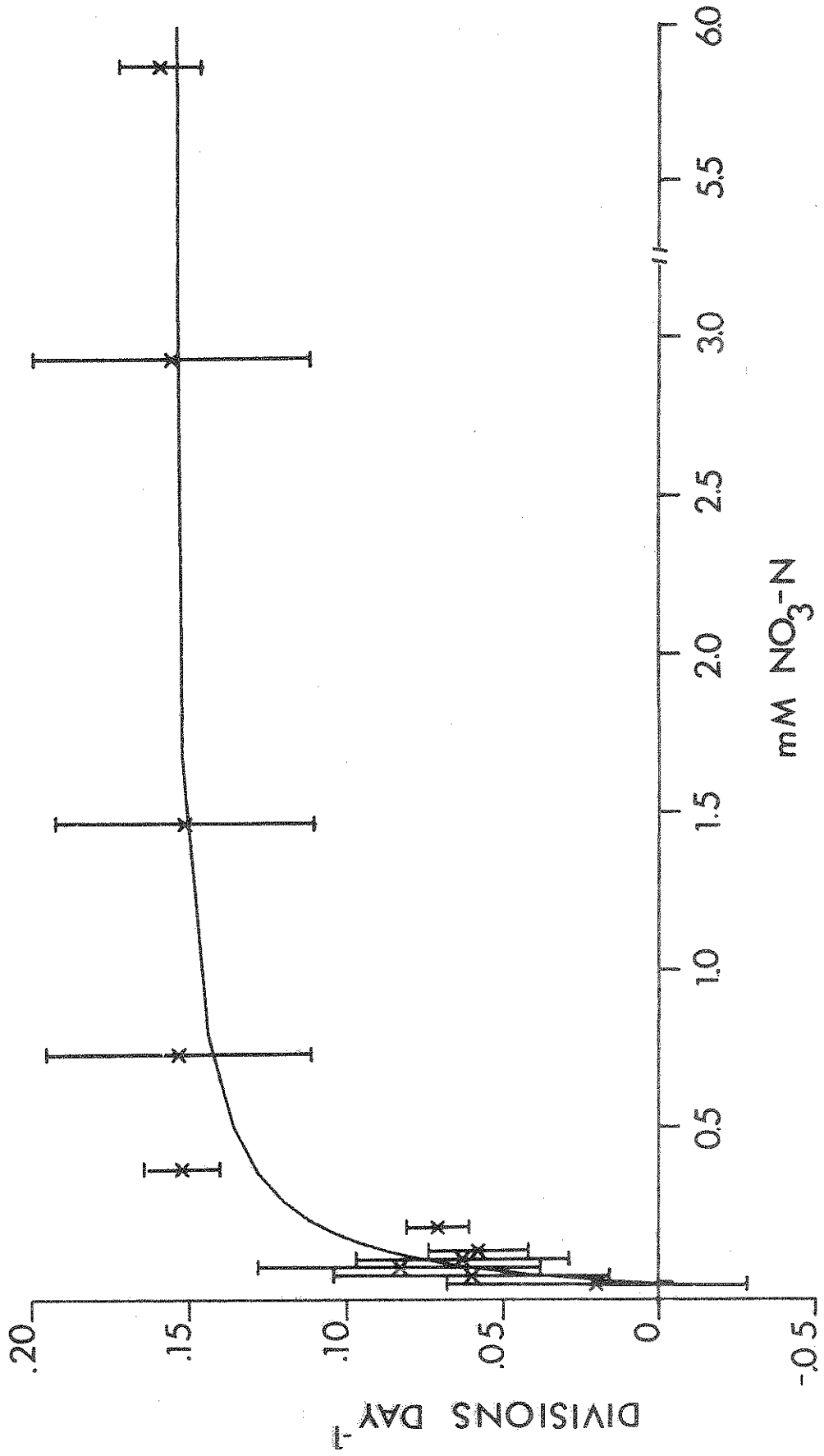


Fig. 42. Pithophora growth versus external nitrate-N concentrations.

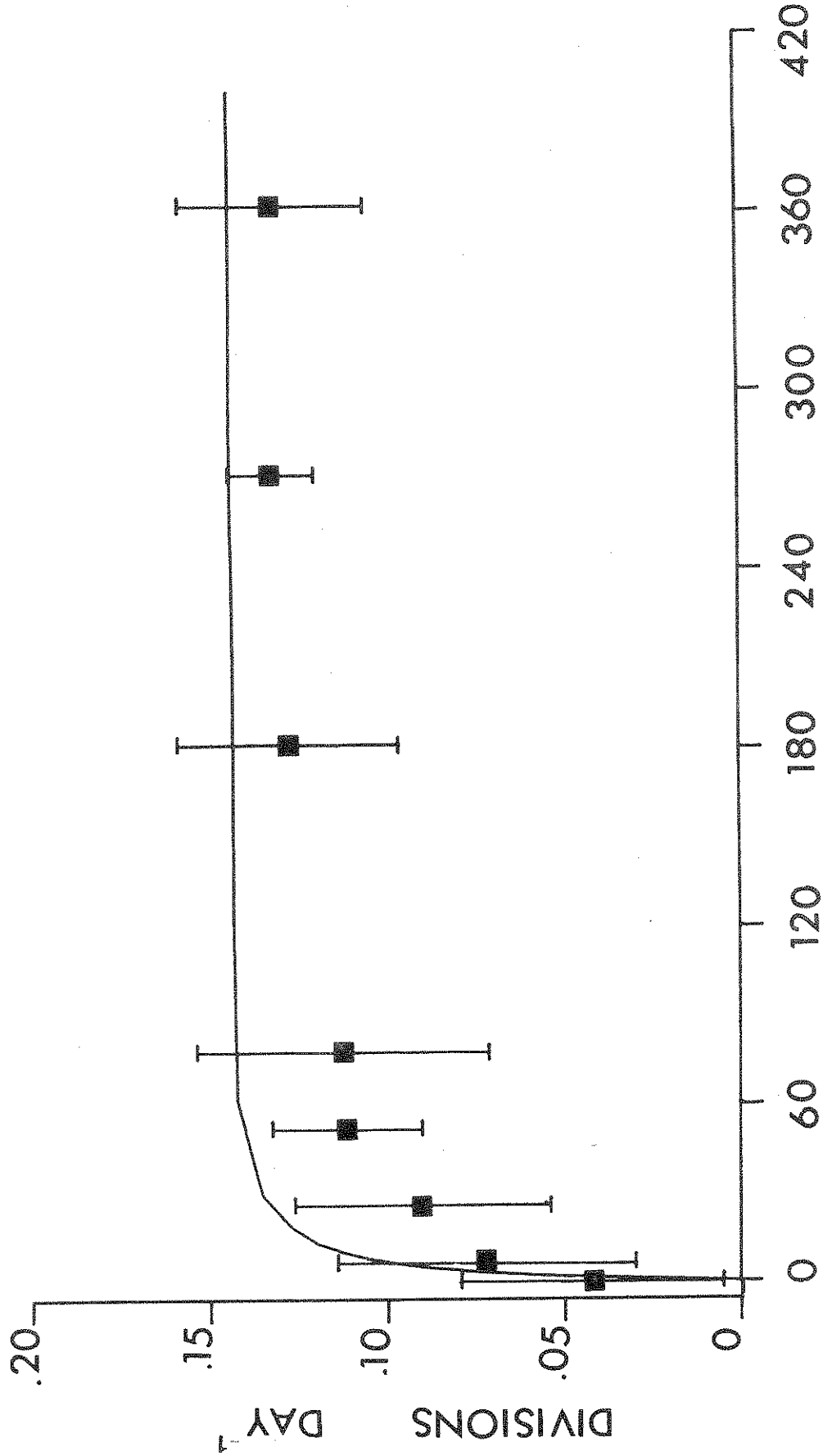


Fig. 43. Pithophora growth versus external phosphate-P concentrations.

Table 22. Kinetic parameters with 95% C.I. for N-limited and P-limited growth of P. oedogonia at 20 C.

Limiting Nutrient	K_s	95% C.I.	V max (Div Day^{-1})	95% C.I.	Sample Size
N	88.3 µM[a]	53-122	.160	.141- .179	95
P	3.2 µM[b]	0.9-5.7	.150	.138- .162	50

[a] 1 µM N= 14.007 µg/l

[b] 1 µM P= 30.974 µg/l

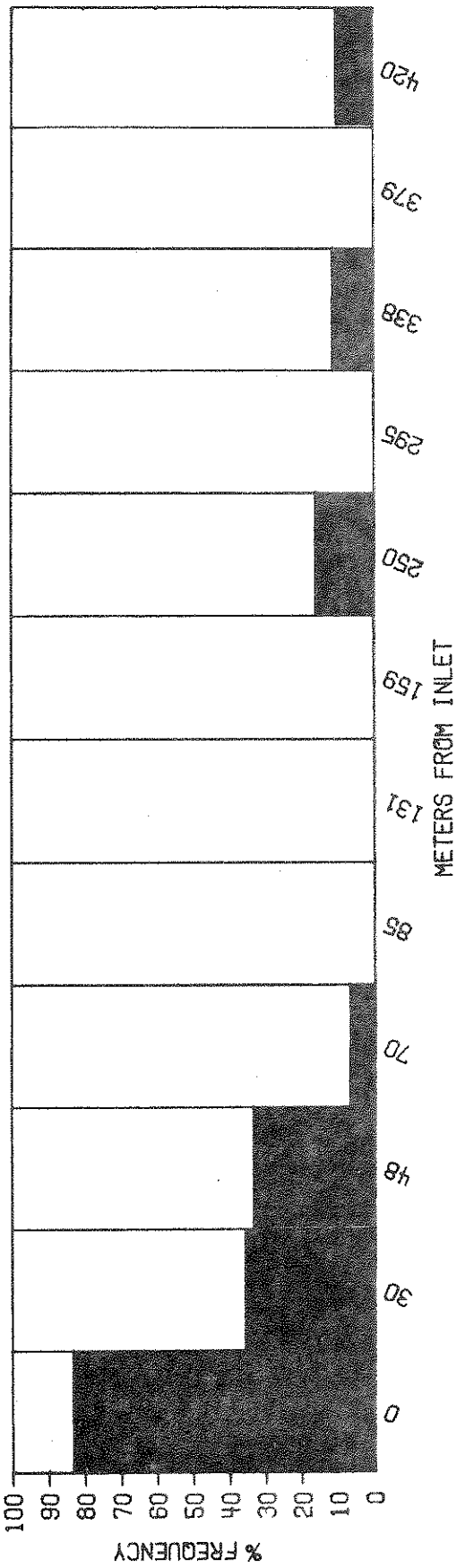


Fig. 44. Frequency distribution of N/P ratios in Surrey Lake, stations 1-12, May 15 - October 7, 1978 and June 26 - September 7, 1979. Black bars indicate N/P ratios>27.6. 0 m = station 1; 30 m = station 2; etc.

their nutritional status especially with respect to nitrogen. The details of the experimental protocol have been described above.

Results of the analysis for alkaline phosphatase which measures the phosphorus nutrition of the alga, indicate very little difference for Pithophora exposed to ambient cove and open water phosphorus concentrations (Fig. 45). According to the criteria suggested by Fitzgerald and Nelson (1966), phosphorus limited algae will have between 5-25 times more alkaline phosphatase activity associated with them than algal cells which have received adequate phosphorus. This is clearly not the case for Pithophora from the cove and lake enclosures, suggesting that phosphorus is generally adequate throughout the lake.

In contrast, the dark ammonia uptake, a measure of nitrogen starvation, shows clear differences between cove and lake exposed algae after fourteen and seventeen days (Fig. 46). The failure to observe differences after a one week exposure period likely reflects utilization of stored nitrogen.

Of particular interest is the data shown for July 21 (Fig. 46). The left 2 bars represent measurements taken on sample material. The right 2 bars represent ammonia uptake by lake collected Pithophora following a 1 h incubation in Cl(II) medium containing 1000 $\mu g\ l^{-1}$ NH_3. It is clear that the ammonia uptake rate declines following preincubation with ammonia for the lake Pithophora but not for the cove Pithophora. This control reinforces the results of the ammonia uptake experiments.

In summary, we have shown that the distribution of Pithophora oedogonia biomass in Surrey Lake is clearly related to the availability of nitrates; i.e., the soluble reactive phosphorus concentration is great enough that nitrate is apparently the limiting nutrient. Although we can now explain the factors that account for the distribution of Pithophora in Surrey Lake, our study can only be of value on a larger scale when used to predict the "susceptibility" of other Indiana lakes to Pithophora infestations. This will be discussed in the next section entitled management considerations.

V. MANAGEMENT CONSIDERATIONS

The intent of this research project was to determine the life history and nutritional requirements of Pithophora in order to establish a basis for sound management recommendations. We have been successful in that our study

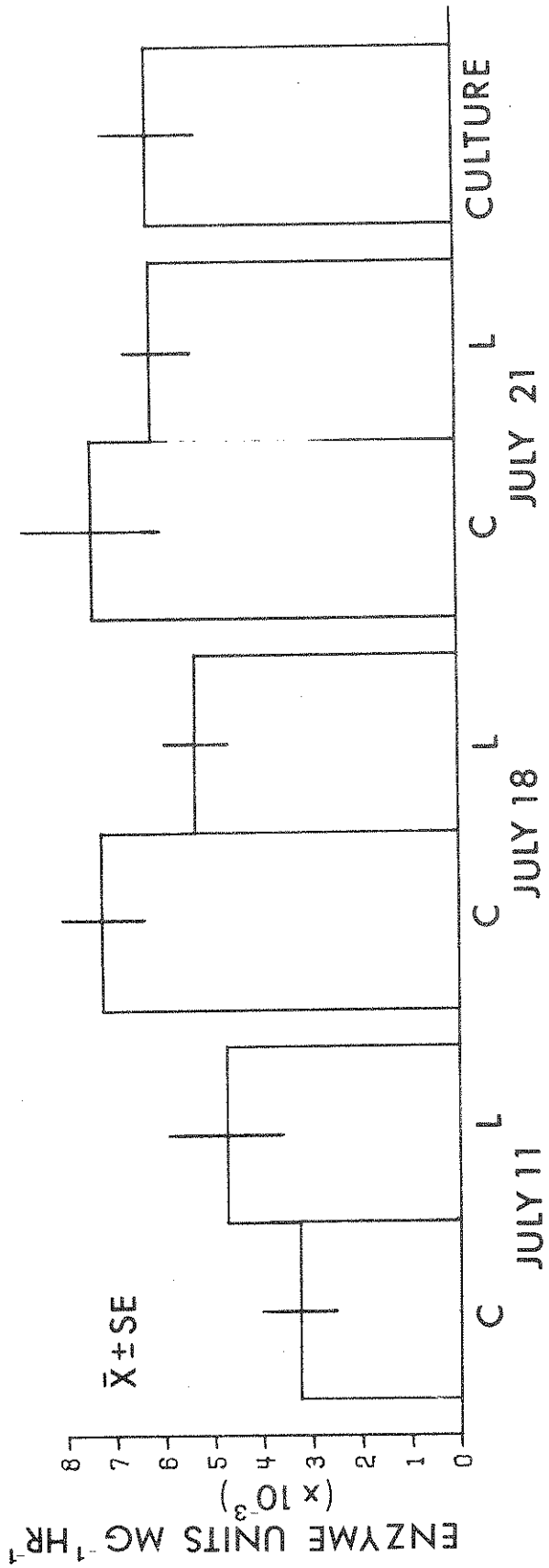


Fig. 45. Alkaline phosphatase activity in filaments collected from cove (C = station 3) and lake (L = station 10) enclosures in Surrey Lake.

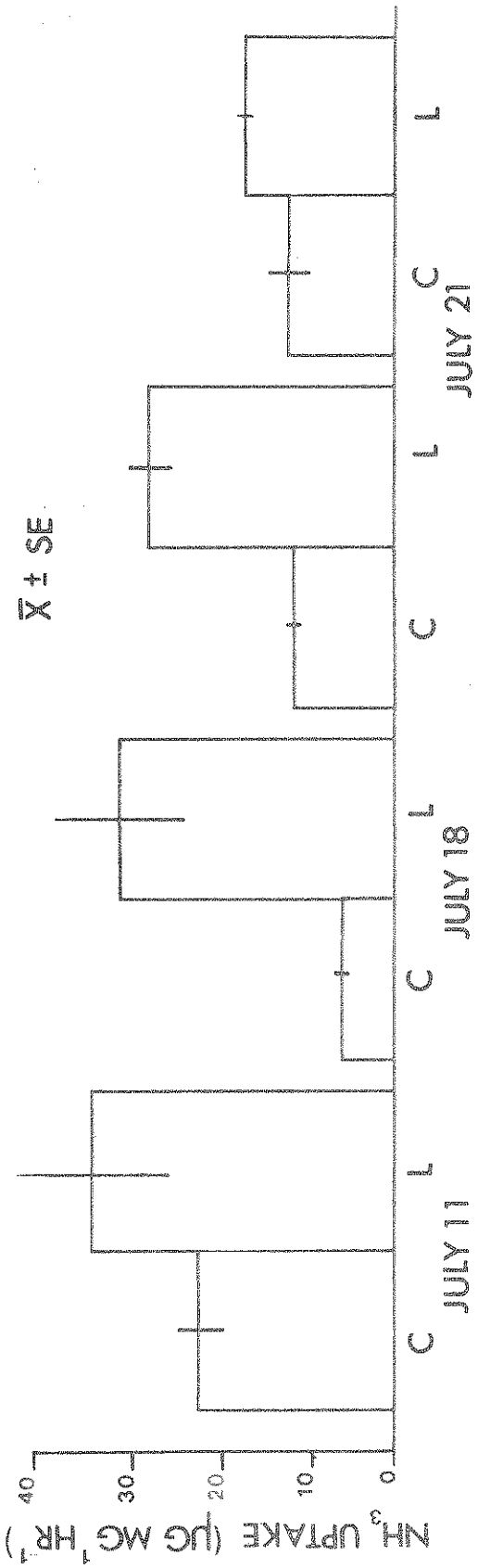


Fig. 46. Dark ammonia uptake in filaments collected from cove (C = station 3) and lake (L = station 10) enclosures in Surrey Lake.

does allow us to make some preliminary suggestions regarding management; however, we would like to emphasize that a considerable amount of field testing, particularly with regard to chemical treatments, remains to be conducted. And as with all research endeavors, more questions than answers have resulted, questions which must be answered in order to develop and strengthen management concepts. Thus, the following recommendations are accompanied by suggestions for further studies, most of which we have already initiated with the continued support of OWRT.

Chemical Treatment

We found that akinetes are more resistant than filamentous cells to copper sulfate treatments. Therefore, if copper sulfate applications are to be effective, initial treatments must be conducted when akinete numbers and, ideally, biomass are lowest. Our studies indicate that this period is at the end of the germination episode for the akinetes in the free-floating mats, i.e., when water temperatures reach 20 C in the spring. However, there are complicating factors to be considered.

For example, the origin of the vegetative biomass that is present in the summer must be determined. Does it develop primarily from overwintering akinetes or from surviving filamentous cells? If from akinetes, is the major source of these akinetes the free-floating mats or the hydrosoil? Our studies indicate that there is tremendous potential for infestation from the hydrosoil since both the numbers and viability of akinetes stored in the upper few cm of hydrosoil are high. This type of information would enable us to determine whether to concentrate chemical treatments on the free-floating mats or on the hydrosoil, possibly with a material that could impregnate the soil and kill the akinetes as they germinate.

Furthermore, we do not know the environmental conditions under which akinetes in the hydrosoil germinate. If they germinate at the same time and under the same conditions as akinetes in the free-floating mats, then treatments at 20 C may be sufficient. However, if the timing in the soil varies (for example, we do know that akinete germination is inhibited under anaerobic conditions), then we must determine those regulating factors in order to best design the protocol for chemical treatments. Another consideration is that of variability in susceptibility to herbicide treatments among different "ages" of Pithophora filaments. In other words, are

germinating akinetes, young rapidly growing filaments, "mature" filaments, or possibly filaments undergoing akinete formation most sensitive to herbicides? And, finally, we must take into consideration the potential of herbicides other than copper sulfate to control Pithophora, compounds such as simazine, diquat, diquat-copper combinations as well as controlled release compounds.

Mechanical Control by Drawdown

Winter drawdowns have been used successfully in the management of a number of vascular aquatic plants but would not be efficaceous in the control of Pithophora. Akinetes remain fully viable after exposure to the prolonged freezing conditions and freeze-thaw periods of an Indiana winter and early spring.

Although akinetes are extremely susceptible to desiccation, they are protected under summer drawdown conditions by a layer of dead cells overlying the thick vegetative mats as well as by frequent rains. Furthermore, the gradual drying out period appears to stimulate the formation of akinetes even more so than in winter, so that any advantages that the presence of akinetes may provide over filamentous cells is quickly enhanced. Certainly, the drawdown of filamentous mats onto the exposed hydrosoil would provide further protection for the numerous akinetes stored in the hydrosoil.

There might be a potential for drawdown if it were conducted in the spring when biomass was relatively low. If the drawdown could be maintained for most of the summer, the mats might be thin enough to dry out completely. However, in heavily infested ponds or lakes, winter and early spring biomass is still high. The free-floating mats do not disappear as does the vegetation of many vascular plants and other algae. In fact, layers of Pithophora vegetation at least 8-10 cm in thickness are often observed on the bottom of Surrey Lake in late winter. In our March, 1980 biomass sampling, the dry wt of Pithophora at station 3 in the shallow end of the lake was 143 g m^{-2} which is comparable to summer biomass figures for both Pithophora and those recorded in the Great Lakes for Cladophora. Thus, it is quite likely that sufficient biomass would be present to protect underlying akinetes throughout the summer. It should also be remembered that April, May and June are the wettest months in Indiana averaging 10.5 cm of rain per month. Although some washout of Pithophora biomass might be expected, the moisture would protect the akinetes and of course, any flood control expected from an impoundment

at this time of year would be lost. If a spring drawdown could be maintained during the summer months, and if the mats and hydrosoil could be disturbed by plowing or other tillage methods, exposure of the akinetes to desiccating conditions might cause significant mortality. However, survival of some of these akinetes plus reestablishment of high nutrient concentrations upon refilling of the impoundment would certainly negate the beneficial aspects of the drawdown.

In conclusion, neither summer nor winter drawdowns appear to be practical solutions for Pithophora control.

Nutrient Limitation

At present, nutrient limitation would appear to offer the greatest potential for the management of Pithophora. We have found P. oedogonia to have very high nitrate-nitrogen and phosphate-phosphorus requirements. The half-saturation constant (K_s) at 20 C relating filament growth to external concentrations of nitrate-N is 1.23 mg l^{-1} (= 88 μM) and for phosphate-phosphorus, 0.1 mg l^{-1} (= 3.22 μM). Lake or pond concentrations of these nutrients higher than or equal to the K_s values would likely support Pithophora growth. Attempts to limit the growth of P. oedogonia in a given body of water should therefore focus on lowering the concentrations of either one of these two nutrients below these values.

We found P. oedogonia growth in the open water portions of Surrey Lake to be limited by nitrate-N. Phosphate-P concentrations at both the shallow (0.112 ± 0.016 mg l^{-1}) and deep water stations (0.083 ± 0.008 mg l^{-1}) were not only similar to one another but also to the K_s value determined through culture studies. Nitrate-N values, however, differed between the two portions of the lake with the concentrations in the deep water area (0.156 ± 0.049 mg l^{-1}) being well below the K_s value determined from culture experiments. Most of the growth of Pithophora (as determined by biomass measurements) occurred in the shallow portion of the lake where nitrate-N levels (0.880 ± 0.241 mg l^{-1}) were high during the summer months.

Like Surrey Lake, the open waters of Indiana's lakes and reservoirs are likely to contain high nitrate-N concentrations, primarily from sediment and agricultural fertilizer runoff. Phosphate-P concentrations, on the other hand, unless enhanced by point sources, would be expected to be much lower, primarily because of the tieup to soil particles, precipitation under

aerobic conditions, and rapid utilization by other algal and vascular plant components. Analyses of samples taken from 27 Indiana lakes (Taylor et al., 1978) revealed mean nitrate-N concentrations of 0.711 $\pm$ 1.017 mg l^{-1} and phosphate-P concentrations of 0.040 $\pm$ 0.068 mg l^{-1}. Thus, nitrate-N values for Surrey Lake as well as the K_s value for N-related growth of P. oedogonia are comparable and well within the standard deviation for nitrate-N values for Indiana lakes. However, the value for phosphate-P from Indiana lakes is much lower than either the Surrey Lake water or K_s values. This comparison leads us to believe that the reason Pithophora is not found to a greater extent in the larger Indiana lakes (at least the open water portions), is because of its high phosphorus requirements. However, channelized areas such as those at Wawasee, shallow coves, and farm ponds which may receive point sources of phosphorus (e.g., from antiquated septic systems, feedlots, lawn fertilizers, etc.) are much more likely to meet the phosphorus requirements of Pithophora and thus be susceptible to infestation.

It is interesting to compare the K_s values for Pithophora with those derived from Gerloff and Fitzgerald's (1976) data for Cladophora. The K_s value relating Cladophora growth to external nitrate concentrations at 23 C is 0.25 mg l^{-1} and to external phosphate-P concentrations, 0.013 mg l^{-1}. Both of these values are lower than those for Pithophora and suggests that one of the reasons Cladophora is of much wider distribution in the United States is because of its lower N and P requirements.

The K_s values for Pithophora were determined at 20 C and 12 μeinsteins $m^{-2}s^{-1}$. We are currently attempting to measure the K_s values at various temperatures and light regimes in order to develop a computer model to predict Pithophora growth under differing environmental conditions. Work is also underway to determine the limiting concentrations of other nutrients and vitamins. However, we realize that much of the present effort in the U.S. in improving water quality is devoted to the elimination of point sources of phosphorus. We support this concept and suggest that, where feasible, phosphorus limitation to below 0.1 mg l^{-1} be considered an essential part of a management program for Pithophora infestations.

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Water Resources Research Center
Lilly Hall of Life Sciences
Purdue University
West Lafayette, Indiana 47907